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THE EXPRESSION OF MATURE BRAIN-DERIVED NEUROTROPHIC FACTOR (mBDNF) ON MALE ALZHEIMER SPRAGUE-DAWLEY RAT TREATED USING HANJELI (Coix lacryma-jobi) LEAVES ETHANOL EXTRACT (14)

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Abstract

Brain derived neurotrophic factor (BDNF) concentration has a relationship with Alzheimer's dementia. BDNF is a neurotropin that has a neuroprotective effect on Alzheimer's disease. The BDNF increase in the area around the lesion is closely related to the progress of nerve function recovery. Coix lacryma-jobi leaf ethanol extract is thought to increase the expression of matureBDNF. The purpose of this study was to observe the effect of ethanol extract of hanjeli leaves on the expression of mBDNF in rat brain cells induced by traumatic brain injury as Alzheimer's disease. This research was design in the form of a post test only control group. The number of samples was 33 mice divided into 3 groups, normal mice without treatment (A), trauma model without hanjeli ethanol extract (B) and trauma model group given oral hanjeli ethanol extract 200 mg per weight (kg) (C) every day for 6 days. In the group C, there was an increase in mBDNF expression by an average of 15.81 ± 2.93 compared to group A and B. This study proved that the treatment of hanjeli ethanol extract per oral would increase the expression of mBDNF in brain cells of rat with Alzheimer's induced by traumatic brain injury.

Keywords: *BDNF, matureBDNF, Alzheimer, Coix lacryma-jobi, ethanol extract*

Introduction

Dementia is a chronic and progressive syndrome that causes impaired neuropsychological function. In this study, the focus of research is dementia of the Alzheimer's type, including memory, thinking, orientation, comprehension, arithmetic, learning, language, and judgmental abilities. Consciousness is not lost and usually followed with a decline in cognitive function, and sometimes preceded by deterioration in emotional control, social behavior, or motivation. This syndrome occurs in Alzheimer's disease, cerebrovascular disease, and other conditions oriented primarily or secondary to the brain (Racine et al, 2017).

The prevalence of Alzheimer's dementia increases with age. Mutations in the following genes cause dominant Alzheimer's dementia of rapid autosomal onset, namely the amyloid precursor protein (APP) gene on chromosome 21, the presenilin-1 gene (PSEN-1) on chromosome 14, and the presenilin-2 gene (PSEN-2) on chromosome 1. These three genes lead to overproduction of amino acid peptide form 42 over amino acid form 40. This results in neuronal death, loss of synapses and formation of NFTs and SPs. The apolipoprotein genotype E ε4 without polymorphisms in other genes was found to be associated with late-onset Alzheimer's dementia. The indicator of this incident is the decreased levels of Brain derived neurotrophic factor (BDNF) in the brain (Evan et al, 2020).

Brain derived neurotrophic factor (BDNF), is a neurotropin that has a neuroprotective effect on ischemic brain injury. The increase of BDNF in the area around the lesion is closely related to the progress of nerve function recovery. However, after ischemic brain injury , there is a decrease in the level of BDNF which results in changes in the ability of neuroplasticity or restoration of nerve cell function, either spontaneously or with rehabilitation induction. Brain derived neurotrophic factor (BDNF) is an important molecule in brain plasticity (Putri et al, 2021).

Brain derived neurotrophic factor (BDNF) plays a role in cell proliferation, differentiation, adhesion and maturation, through specific receptor protein tyrosine kinase (PTK), which induces growth, survival and regeneration of central nervous cells after injury. The previous studies reported that after induction of BDNF in vitro, the differentiation of neural stem cells (NSCs) was increased twofold. BDNF has strong activity in the proliferation, differentiation and activation of endogenous and exogenous NSCs (Sari et al, 2018).

Hanjeli (Coix lacryma-jobi) has a higher protein, fat, and vitamin B1 content than other cereal crops. Its leaf contains alkaloids, carbohydrates, saponins, glycosides, flavonoids, phenols, tannins, and steroids (Zhang et al, 2019). Ethanol extract from hanjeli leaves contain compounds that have antioxidant, antibacterial, anti-inflammatory, antiviral and anticancer activities (Diningrat et al., 2020).

Various studies have shown that bioactive compounds from hanjeli ethanol extract have antioxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic effects. While in vivo study, the ethanolic extract of hanjeli has been shown to be able to penetrate the blood brain barrier and maintain high biological activity in the brain. The pleiotropic molecules found in hanjeli ethanol extract are strong and can interact with a number of molecular targets (Son, 2019).

Based on research by Devaraj (2020), animal studies on rats treated with head injury and given hanjeli ethanol extract were able to improve cognitive function and brain plasticity. The treatment through hanjeli ethanol extract decreased carbonyl protein levels which would increase after injury, thereby showing the potential of the bioactive compounds that present in the hanjeli ethanol extract as free radical scavengers in reducing oxidative damage and Alzheimer's pathology. Possibility of antioxidant activity of hanjeli ethanol extract was obtained from the structure of two electrophilic groups α, β-, -unsaturated carbonyl which can react on nucleophiles such as glutathione. The bioactive compounds of its extract also have the potential to increase BDNF, inhibit lipid peroxidation, and neutralize the reactivation of oxygen and free radicals from nitric oxide. The characteristics of the bioactive compound of hanjeli ethanol extract are supported by the ability to cross the blood brain barrier so that it can provide direct protection against neurons (Seo et al, 2009).

Based on this background, this study aims to (1) prove that the administration of head trauma which is assumed to be the induction of Alzheimer's dementia causes changes in mBDNF expression in rat brain cells. It proves that the treatment of bioactive compounds of hanjeli ethanol extract can increase mBDNF expression in rats with traumatic brain injury (alzheimer's dementia).

Methods

This research was an experimental laboratory analytical test on experimental animals with a post-test only control group design. This design allowed researchers to measure the effect of treatment (intervention) on the experimental group by comparing the experimental group with the control group. However, this design did not allow the researcher to determine the extent of change, because the test was carried out at the end of the treatment (not to determine the initial data) (Krisnan, 2021).

Group A (six days) -> O1

Group B (six days) ->O2

Group C (six days) ->O3

Information :

Group A : Negative control

Group B: The group that was given a traumatic brain injury

Group C: The group given traumatic brain injury and ethanol extract of hanjeli leaves

O1 : Observation group A after 6 days

O2 : Observation group B after 6 days

O3 : Observation group C after 6 days

Population and sample

The experimental animals used in this study were male Sprague-Dawley rats, aged 2.5-3 months, body weight 280-320 grams, healthy and obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. The selection of mice as experimental animals was based on the consideration that Sprague-Dawley mice are genetically similar to human and have the ability to adapt to the laboratory environment (Sukriani & Arisani, 2020).

The sample allocation (grouping) used simple random by numbering each rat firstly. Therefore, experimental animals, experimental sites, and other research materials can be said to be homogeneous (Krisnan, 2021).

Place and time of research

The maintenance of experimental animals during the study was carried out in experimental animal cages at the Multifunction Laboratory of UIN Ar-Raniry Banda Aceh. The examination of brain tissue preparations was carried out at the Biology Study Program, UIN Ar-Raniry Banda Aceh.

The research was carried out for 2 (two) months, consisting of the stages of material preparation and tools, treatment, inspection, and preparation of report. The treatment of experimental animals was carried out for 1 day. Then, the brain tissue preparations were examined on the 7th day in the form of the number of cells expressing mBDNF in the contused cerebral cortex of male Sprague-Dawley rats, with a 1000x microscope. The Positive cells were counted in 20 fields at each sample. Observations of mBDNF expression peaked after 24 hours in the contused cerebral cortex, and decreased to a point of equilibrium after the first 24 hours in the penumbra area of injury. Treatment of research subjects: traumatic brain injury was given to two experimental groups (Groups B and C) once and after that the research subjects were given ethanol extract of hanjeli leaves 200 mg/kg orally every day for 6 days.

Data collection Techniques

Rat selection

The experimental animals were 33 healthy male rats, looked active, aged 2.5-3 months, and body weight 280-320 grams. All of these mice were numbered on their fur, which would be used in a simple randomization process. Then, the researcher made a fold of paper containing the number of each rat and carried out a lottery process to divide the rats into 3 treatment groups with the number at 11 each.

Rat acclimatization

All rats were then adapted to laboratory conditions for 7 days in order to adjust with the environment and during adaptation, they were fed BR I .

Research process

At the beginning of the study (2nd day) group A (negative control) was sacrificed with cervical dislocation, and then brain organs were taken for making immunohistochemical preparations. Group B (positive control) on day 1 was given brain injury treatment, observed and treated for 6 days. Then the mice were sacrificed on the 7th day with cervical dislocation after anesthesia with ether. Next, the brain organs were taken for making immunohistochemical preparations.

Group C from day 1 was treated with brain injury and ethanol extract of hanjeli leaves on turmeric extract (200 mg/kg orally every day for 6 days. Then the mice were sacrificed on day 7 with cervical dislocation after anesthesia with ether. Then, the brain organs were taken for the manufacture of immunohistochemical preparations.

Brain injury treatment

The modification of Feeney Traumatic Brain Injury (TBI) weight drop model (Weissenberger, 2010) was performed with the way male Sprague-Dawley rats are shaved and scrapped 4 mm on the right frontoparietal to obtain bone exposure. The center of the skin opening was at 1.5 mm posterior to the bregma and 2.5 mm lateral from the midline, a load weighing 40 grams dropped to intact bone from a height of 1 meter with a load diameter of 2.5 mm. Immediately after head injury, the scalp was closed with sutures (Silk 3.0 cutting) and the animals are returned to the cage (Troubat et al, 2021).

Brain removal

On the 7th day, the rats in groups A, B and C were killed with cervical dislocation after implementing anesthesia with ether and the surgical removal of mice brain.

Immunohistochemical method of mBDNF marker

The slides were washed with PBS pH 7.4 once for 5 minutes. Blocking endogenous peroxide needed 3% H_2O_2 for 15 minutes. Then, Wash by using PBS pH 7.4 three times, each for 3 minutes. Unspecific protein blocking took 5% FBS containing 0.25% Triton X-100. Next, Wash using PBS pH 7.4 three times, each for 3 minutes. Incubation took mBDNF antibody for 60 minutes. After that, Wash using PBS pH 7.4 three times, each for 3 minutes. Incubation took anti mouse biotin conjugated for one hour at temperature room. Subsequently, Wash using PBS pH 7.4 three times, each for 3 minutes. Incubation took SA-HRP (Strep-Avidin Horse Radis Peroxidase) for 10 minutes. Moreover, Wash using PBS pH 7.4 three times, each for 3 minutes. Drop with DAB (Diamino Benzidine) and incubate for 5-15 minutes. Then, Wash using PBS pH 7.4 three times, each for 5 minutes. Counterstaining took Mayer Hematoxylen which was incubated for 1 minute and washed using tap water. Rinse with dH_2O and air dry. Mounting needed entelan and closed using a glass cover. At last, Observe on a light microscope.

Counting mBDNF cells in cerebral cortex contusions

The Expression of molecules that were positive with primary antibodies would appear brown in 1000X light microscopy. The calculated cerebral cortical contusion cells were located in the cerebral cortex area. The positive cells were counted in 20 visual fields (HPF) in each sample.

Data processing and analysis

The results obtained were analyzed using the Shapiro Wills normality test. If the data is normally distributed, then statistical analysis will be carried out by testing the ANOVA hypothesis (analysis of variance). However, if the data is not normally distributed, the Kruskal Wallis hypothesis will be carried out. If there is a difference, then the analysis will continue with the Post Hoc Tukey test to find out different pairs of data (to see the differences in each group). This study means that if the p value <0.05.

Results

This study used 33 experimental rats that met the predetermined criteria. Experimental mice were divided into 3 groups. Each group consisted of 11 rats. The first group was the group that was not

given any treatment (Group A). The second group was a group of mice that were treated with traumatic brain injury (Group B). The third group was a group of mice who were given the treatment of traumatic brain injury and turmeric extract (Group C). All mice were observed after 24 hours post-traumatic brain injury treatment. Observations were made by sacrificing rats with cervical dislocation as well as the help of anesthesia. The procedure of this research had gone through an ethical test. The mouse brain organs were taken for process of immunohistochemical preparations. Immunohistochemical examination was carried out to observe cells on mBDNF due to the treatment given

Expression of mBNDF in Brain Tissue Treatment Group

From the results of the examination of brain tissue by using immunohistochemical staining techniques with mBDNF primary antibody, it was found that there was a change in mBDNF expression in each treatment group.

Table 1. mBDNF Expression Based on Treatment Group

Treatment group	Average
Group A	6.85 ± 1,35
Group B	11.65 ± 1,70
Group C	15.81 ± 2,93

To determine the difference in mBDNF expression, the Tukey HSD test was performed. The results of the immunohistochemical examination of brain tissue showed that the brain tissue of the control group showed an average amount of mBDNF expression of 6.85. The treatment of traumatic head injury and hanjeli leaves ethanol extract (group C) in experimental animals significantly increased the average mBDNF expression in group A (p=0.0001) and group B (p=0.0001) to 15.81.

It could be concluded that in the treatment of head injury and hanjeli leaves ethanol extract (Group C), mBDNF expression increased when compared to the negative control group (Group A) and the group given head injury only (Group B). The following was a histological picture of mBDNF expression using Immunohistochemical staining and an Olympus BX 50 microscope with 1000x vision enlargement (Figure 1).

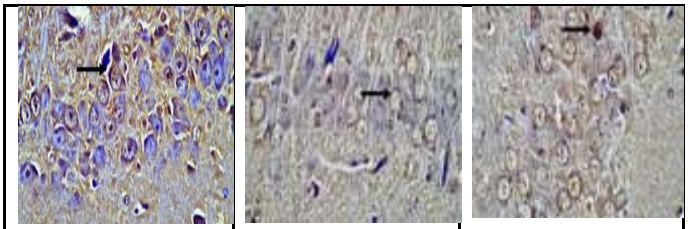

Figure 1 The comparison of mBDNF expression

Discussion

Alzheimer's Dementia

Alzheimer in this study is the early symptoms induced by inflicting a head injury on mice. The head injury induces a complex series of immunological or inflammatory tissue responses which is similar to the ischemic reperfusion injury conditions that often occur in people with Alzheimer. The primary and secondary injury activates the release of cellular mediators including proinflammatory cytokines, prostaglandins, and free radicals. This process induces chemokines and adhesion; molecules and immunity mobility; and glial cells in synergistic and parallel patterns. Infiltration of leukocytes into the tissue is mediated through an increase of cellular adhesion molecules such as P-selectin, intracellular adhesion molecules (ICAM-1), and Vascular adhesion molecules (VCAM-1) in response to the inflammatory process. The damaged tissue would be eliminated and astrocytes would produce microfilaments and neutrophils to synthesize scar tissue. Pro-inflammatory enzymes such as tumor necrosis factor, interleukin 1-B, and interleukin-6 are elevated within hours after injury happened. The progression of tissue damage is directly related to neurotoxic mediators and indirectly to the release of NO and cytokines (Yasmin et al, 2021).

The increase in free radicals is another contributing factor in the metabolic crisis caused by head injury. This free radical component is very reactive because it gets electrons and the surrounding tissue which causes damage toward cell membranes, proteins, and DNA. There are two main types of free radicals, namely reactive oxygen species (ROS) and reactive nitrogen species (RNS).

The production of ROS levels in normal metabolic activity can be well controlled by the cellular antioxidant defense system. However, after a head injury, ROS levels are produced in excess and cause oxidative damage. Mitochondria are a source of formation of free radicals. During normal metabolism, Tricarboxylic acid cycle (TCA) produces an equalizer to produce ATP. However, in head injury, the availability of this equalizer is lost and O2 production is increased. Simultaneously, head injury induces Ca2+ accumulation and can activate various enzymes including xanthine dehydrogenase, phospholipase A2 and nitric oxide synthase (NOS) thereby increasing O2- and NO production (Mountaki et al, 2021).

The production of free radicals increases significantly after a head injury. In severely injured mice, hydroxyl radical (OH) production increased by 60% in the first minute and reached a peak 30 minutes after trauma and then decreased again. Meanwhile, NOS production increased very rapidly with peak levels in the fifth minute and returned to normal after 6 hours after trauma. Apart from direct formation after trauma, there is indirect evidence of lipid peroxidation, protein nitration, and DNA oxidation. Lipid peroxidation occurs when ROS react with polyunsaturated fatty acids, causing changes in membrane permeability. Protein oxidation or nitration products increased significantly after 30 minutes of trauma, but what is interesting is that the levels of oxidized proteins is inversely reversed with the severity of head injury (Birla et al, 2020).

DNA damage due to free radicals can occur, which is indicated by the activation of DNA repair enzymes. Poly-ADP ribose polymerase (PARP) is the repair enzyme of nuclear DNA that consumes NAD+ and its activity has been shown increasing after several types of head injury. Pathological activation of PARP has been proved reducing cytosolic NAD+ levels to inhibit the breakdown of glucose and decrease ATP production. Glutathione peroxidase (GPx) activity is also increased after head injury within 24 hours (Phadke et al, 2021).

The nomenclature related to cell death phenotypes is confusing, but one classification based on morphological analysis of cell death identifies three types of cell death. Type 1 is manifested by nuclear condensation and pyknosis, reduction of cytoplasmic volume, cell fragmentation, and phagocytosis. Type 2 or autophagic degeneration is characterized by substantial autophagic vacuolization in the cytoplasm. Type 3 or cytoplasmic cell death is characterized by complete disintegration and removal of the organelle. The incidence of type 1 cell death is associated with apoptosis, while types 2 and 3 are associated with necrosis (Wang et al, 2020).

Necrosis is characterized by loss of membrane integrity and cellular swelling, damage to organelles, lysosomal rupture, and uncontrolled cell lysis that often leads to tissue inflammation. In contrast, apoptosis is characterized by intact membrane integrity and cell shrinkage, condensation of the plasma and nucleus, and blebbing of the plasma membrane. In the late stages of apoptosis, cells may undergo disintegration into apoptotic bodies that will be phagocytosed by surrounding cells and do not cause an inflammatory response (Li et al, 2020). Apoptosis and necrosis may be induced by the same cause, but the severity of the cause determines where cells undergo one of the alternative pathways of cell death. Intracellular ATP levels are an important factor in initiating apoptosis or necrosis (Wang et al, 2020).

Brain Derived Neurotrophic Factor (BDNF)

Brain Derived Neurotrophic Factor (BDNF) is one of the neurotrophic factors that supports the differentiation, maturation, and survival of neurons in the nervous system and exhibits neuroprotective effects under adverse conditions, such as glutamatergic stimulation, cerebral ischemia, hypoglycemia, and neurotoxicity. BDNF stimulates and controls the growth of new neurons from neural stem cells (neurogenesis), wherein BDNF can be identified in most areas of the brain including the olfactory bulb, cortex, hippocampus, basal forebrain, mesencephalon, hypothalamus, brainstem and spinal cord. BDNF levels are decreased in many neurodegenerative diseases such as Parkinson's disease, multiple sclerosis (MS) and Huntington's disease. In addition to its neuroprotective effects, BDNF plays a major role in homeostasis (Hutten et al, 2020). Brain Derived Neurotrophic Factor (BDNF) is a member of the

neurotrophin growth factor family along with nerve growth factor (NGF); neurotrophins-3 (NT-3), NT4/5 and NT-6. BDNF is synthesized in the endoplasmic reticulum as a precursor protein with a molecular weight of 32-35 kDa (pro BDNF) that travels through the Golgi apparatus and trans-Golgi networks. The terminal domain of pro-BDNF is cleaved by different convertase protein enzymes to form the active BDNF (mBDNF) with a molecular weight of 13 kDa (Sugasini et al, 2020).

mBDNF Function

Neurogenesis

One of the earliest identified functions of mBDNF is its role to promote the survival of peripheral neurons during brain development. It was reported that the exogenous treatment of mBDNF resulted in an increase in dendritic length and complexity of pyramidal neurons in developing visual cortex with many layers. This suggests that BDNF not only enhances 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 the increase in dendritic growth elicited by exogenous BDNF, indicating that neurons must be sufficiently active to respond to the growth-enhancing action of BDNF (Namgyal et al, 2020).

In a central nervous system trauma model, by using adult mouse retinal ganglion cells, it was observed that in-vivo injection of BDNF enhances neuronal survival by activating the TrkB, MAPK and PI3K-PKB pathways as well as inhibiting caspase-3-induced apoptosis. Neurogenesis in the hypothalamus is enhanced by the continuous long-term treatment of BDNF for 12 days (Namgyal et al, 2020).

Synaptic plasticity

BDNF is involved in the regulation of synaptic plasticity by pre- and post-synaptic mechanisms. BDNF is essential for pre-synaptic vesicles, which depend on activation of the NMDA receptor (N-methyl D-aspartate) on cultured neocortical neurons from BDNF-deprived mice. The role of BDNF as a paracrine (retrograde messenger) was known recently, where treatment of BDNF to the hippocampal region was able to restore spinal polymerization and more stable long-term potentiation on mice. BDNF not only increases NMDA levels and intracellular calcium concentrations but also liberates Mg2+ NMDA receptors and promotes long-term changes in synaptic activity. The reduction of TrkB and BDNF secretion reduces the induction of Long term potentiation (LTP). Thus, BDNF is involved in NMDA receptor traffic by increasing calcium influx, leading to post-synaptic release of BDNF which, in turn, increases pre-synaptic vesicles, and increases LTP and synaptic plasticity (Rana et al, 2020).

Role of BDNF in Neurological Disorders

The ability of BDNF to augment neurogenesis and enhance synaptic plasticity, as discussed above, implies that this compound may have a role in several neurological conditions such as Alzheimer disease, dementia and autism. In fact, it is suspected that decrease of BDNF serum levels may have a role in the pathophysiology of cognitive deficits seen in patients with type 2 diabetes mellitus (Colucci-D'Amato et al, 2020). BDNF levels were significantly lower on patients with schizophrenia who had lower cognitive scores than controls. This suggests that BDNF may be involved in the pathophysiology of schizophrenia, and associated cognitive impairment, particularly direct memory. In fact, it is suspected that BDNF is a compound that links depression and type 2 diabetes mellitus.

It is known that depression is a risk factor for the development of type 2 diabetes mellitus, while most patients with type 2 diabetes mellitus also have depression. This led to the suggestion that BDNF may play an important role in linking depression and type 2 diabetes mellitus. In this context, it is interesting to note that chronic alcohol consumption may exacerbate type 2 diabetes mellitus and decrease BDNF levels, implying that alcohol-induced peripheral neuropathy, dementia and decreased cognitive function may also be due to low levels of BDNF (Colucci-D'Amato et al, 2020).

Decreases in GABAergic (gamma-aminobutyric acid) neurons in the substantia nigra of mice have been observed in Huntington's disease, and may also occur in Parkinson's disease. BDNF is known to prevent excitotoxic killing of cultured GABA neurons. Continuous infusion of supranigral BDNF

for 3 weeks prevented nigral neuronal loss caused by ibotenic - induced acid destruction of the putamen caudatum and globus pallidus. BDNF increased nigral neuron size by 25%. This effect was found specifically for the receptor tyrosine kinase TrkB which mediates the actions of BDNF. These results suggest that exogenously given BDNF may prevent neuronal loss, and reduce the susceptibility of nigral neurons to glutamate input, and thus may be beneficial in Huntington's and Parkinson's disease (Namgyal et al, 2020).

In this context, it is important to note that the Huntingtin protein, which is a 350-kilodalton molecular weight protein, is mutated in Huntington's disease. The mutant protein is assumed to acquire a toxic function that harms striatal neurons in the brain. The loss of this beneficial activity of native huntingtin can also lead to striatal neuronal death, leading to the development of Huntington's disease.

It was reported that wild-type huntington up regulates the transcription of BDNF – pro-survival factor produced by cortical neurons that is necessary for the survival of striatal neurons in the brain. The beneficial activity of huntingtin is lost when the protein is mutated, resulting in decreased cortical BDNF production. This results in a lack of neurotrophic support for striatal neurons, which then die. Furthermore, it was noted that the decreased expression of BDNF observed in Huntington's disease exacerbates dopaminergic neuronal dysfunction, which may participate in the motor impairment associated with this neurodegenerative disorder. These results are supported by reports of reduced TrkB receptor expression occurring in mouse models of Huntington's disease and in the human brains of post-mortem patients who died from this disease (Colucci-D'Amato et al, 2020).

Bioactive Compounds of Hanjeli Leaf Extract

Hanjeli is a plant that has many benefits. Almost all parts of the Hanjeli plant such as seeds, leaves, stems, and roots can be utilized (Yu et al, 2021). In previous studies, the results of phytochemical screening of Hanjeli leaves showed the presence of alkaloids, saponins, glycosides, flavonoids, phenols, tannins and steroids as the main constituents responsible for antibacterial (Yu et al, 2021). There are 49 phytochemical compounds in hanjeli leaf extract. Of 49 phytochemical compounds in the leaves, 33 compounds have been known as bioactivity. Of 33 phytochemical compounds in leaves, their bioactivity is known to act as genotoxins, carcinogenic agents, mutagens, and inducers of apoptosis, anti-inflammatory, therapeutic agents, antioxidants, metabolites in humans, human urine, plants, algae, mice, Escherichia coli, and Saccharomuces cerevisiae, reproductive sterility in organisms, osmolytes, antitumors, biomarkers, human xenobiotics, antilipemics, B vitamins, pheromones, substances flavoring, antifungal, antibacterial, allergen, antimicrobial, catecholamines, cholestanoid and enterolactone lignans (Diningrat et al, 2020).

A study from Seo et al in 2009 proved that the treatment of ethanol extract of hanjeli leaves given routinely for 6 weeks can improve insulin resistance and normalize hyperglycemia, and then there is also a decrease in carbonyl levels. Thus, providing ethanol extract of hanjeli leaves can reduce the rate of oxidative stress in the brain and trigger an increase in BDNF expression in the frontal cortex and hippocampus (Sarkar, 2020).

A research by Hu et al (2020) suggested that the ethanolic extract of hanjeli leaves was also able to increase mitochondrial biogenesis and suppress the release of various proinflammatory cytokines such as interleukin 6 and tumor necrosis factor alpha (TNFalfa). All of these channels put the role of hanjeli leaves ethanol extract as an antioxidant, anti-inflammatory, antiapoptotic and neuroprotector.

The research on experimental animals rats treated with head injuries and given ethanol extract of hanjeli leaves in turmeric extract was able to improve cognitive function and brain plasticity. The treatment of hanjeli leaves ethanol extract reduced the protein carbonyl levels which increased after injury, thereby demonstrating the potential of hanjeli leaves ethanol extract as a free radical scavenger in reducing oxidative damage and Alzheimer's pathology. Possibly the antioxidant activity of hanjeli leaves ethanol extract is derived from the structure of two electrophile groups α, β-unsaturated carbonyl which can react to nucleophiles such as glutathione. Hanjeli leaf

ethanol extract also has the potential to increase BDNF, inhibit lipid peroxidation, neutralize oxygen reactivation and free radicals from nitric oxide. The characteristic of the ethanolic extract of hanjeli leaves is supported by the ability to cross the blood brain barrier so that it can provide direct protection against neurons (Tsay et al, 2021).

Another study in experimental animals TLR4-/- mice compared with wild type given intraperitoneal injection of hanjeli leaf ethanol extract after being given a contusion injury with a load impact model on the head resulted in minimal cerebral edema and improved neurological function. The value of neurological deficit was significantly lower in the group given the injection of hanjeli leaf ethanol extract. The ethanolic extract of hanjeli leaves also reduces neuronal damage induced by lipopolysaccharide (LPS) (Hu et al, 2020).

In this study, the effect of hanjeli leaf ethanol extract was investigated orally on mBDNF expression after traumatic brain injury. This research was an experimental animal laboratory study. The increase in mBDNF in the area around the lesion was closely related to the progress of nerve function recovery. However, after ischemic brain injury, there was a decrease in the level of mBDNF which resulted in hanges in the ability of neuroplasticity or restoration of nerve cell function, either spontaneously or by rehabilitation induction. mBDNF is an important molecule in brain plasticity (Sari et al, 2018).

Mature Brain Derived Neurothrophic Factor (mBDNF) played a role in cell proliferation, differentiation, adhesion and maturation, through specific receptor of protein tyrosine kinase (PTK), which induced growth, survival and regeneration of central nervous cells after injury. The study reported that after induction of mBDNF in vitro, the differentiation of NSCs increased twofold. mBDNF had strong activity in the proliferation, differentiation and activation of endogenous and exogenous NSCs (Evana et al, 2020).

Hanjeli leaf ethanol extract had the potential to increase mBDNF, inhibited lipid peroxidation, and neutralized the reactivation of oxygen and free radicals from nitric oxide. This characteristic of hanjeli leaves ethanol extract was supported by the ability to cross the blood brain barrier so that it could provide direct protection to neurons. From the results of the study, intranasal tratmentof hanjeli leaf ethanol extract significantly increased mBDNF expression ($p = 0.0001$) compared to the negative control group and the head injury treatment group. There was a significant decrease in mBDNF expression ($p = 0.0001$) in the head injury treatment group compared to the negative control.

The activation of BDNF (ligand) from tyrosine residues resulted in the activation of different intracellular pathways, leading to neural plasticity, neurogenesis, stress resistance and cell survival. This suggested the comparative flexibility of the Trk receptor in terms of pro-survival function. Thus, mBDNF signaling channel activated one or both transcription factors CREB and CREB-binding protein (CBP) that regulated the expression of genes encoding proteins involved in neural plasticity, stress resistance and cell survival (Rana et al, 2020).

In a study by Namgyal et al (2020) mBDNF was involved in the regulation of synaptic plasticity by pre- and post-synaptic mechanisms. mBDNF was essential for pre-synaptic vesicles, which depended on activation of the NMDA receptor (N-methyl D-aspartate) on cultured neocortical neurons from BDNF-deprived mice. The role of BDNF as a paracrine (retrograde messenger) has been known recently, where the treatment of BDNF to the hippocampus was able to restore spinal polymerization and more stable long-term potentiation in mice. BDNF not only increases NMDA levels and intracellular calcium concentrations but also released $Mg2+$ NMDA receptors and promoted long-term changes in synaptic activity. The reduction of TrkB and BDNF secretion reduced LTP induction. Thus, BDNF wss involved in NMDA receptor traffic by increasing calcium influx, leading to post-synaptic release of BDNF which, in turn, increased pre-synaptic vesicles, LTP and synaptic plasticity.

The role of hanjeli leaf ethanol extract in increasing BDNF expression was related totranscription of the gene encoding BDNF. hanjeli leaves ethanol extract has been proven to increase levels of cAMP and ERK (extracellular signal regulated kinases) as well as p38 kinase. The cAMP compound would activate Protein Kinase A (PKA),

an enzyme needed to increase the activity of cAMP-response element binding (CREB) and protein of (CREB). This activated CREB would then occupy the promoter of the gene encoding BDNF and then started the gene transcription process which caused the production of more BDNF protein (Colucci-D'Amato et al, 2020).

The Research on experimental animals (mice) treated with head injury and given hanjeli leaves ethanol extract in turmeric extract was able to improve cognitive function and brain plasticity. The treatment of ethanol extract of hanjeli leaves reduced the levels of protein carbonyl which increased after injury, thereby showing the potential of hanjeli leaf ethanol extract as a free radical scavenger in reducing oxidative damage and Alzheimer's pathology. The possible antioxidant activity of hanjeli leaves ethanol extract was derived from the structure of two groups of -electrophile α, β-unsaturated carbonyl which can react with nucleophiles such as glutathione (Sugasini et al, 2020).

Hanjeli leaf ethanol extract also has the potential to increase BDNF, inhibit lipid peroxidation, and neutralize the reactivation of oxygen and free radicals from nitric oxide. This characteristic of hanjeli leaf ethanol extract is supported by the ability to cross the blood brain barrier so that it can provide direct protection to neurons (Colucci-D'Amato et al, 2020; Sarkar, 2020).

The new finding from this study is that the treatment of hanjeli leaf ethanol extract on Intraoral turmeric extract increased mBDNF expression in brain cells with traumatic head injury, which is made by modulating the TrkB system to increase PI3K and ERK expression. PI3K plays a role in the antiapoptotic pathway, while ERK is in neuroplasticity.

Based on the presented research results and according to the research objectives, it can be concluded that:

1.The oral treatment of hanjeli leaf ethanol extract on turmeric extract increased the expression of mBDNF in brain cells with traumatic head injury.

2.There was a significant difference in mBDNF expression among the negative control group (Group A), the positive control group (Group B) and the treatment group (Group C).

Suggestion

Based on the results of the presented research, there are some suggestions that should be considered as follows:

1.Further research is needed to see the expression of mBDNF with serial times of 24 hours, 72 hours, and 7 days after treatment.

2.Further research is needed on various other variables that have not been observed by researchers, such as TRkB, PI3K, PKA, ERK, VEGF, pro-BDNF/p75NTR

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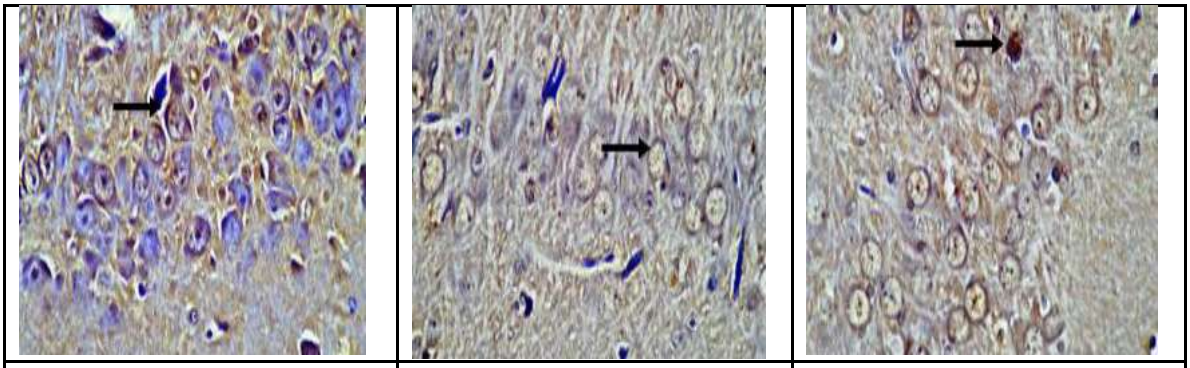
Table 1. mBDNF Expression Based on Treatment Group

Treatment Group	Average
Group A	6.85 ± 1,35
Group B	11.65 ± 1,70
Group C	15.81 ± 2,93



Control Negative	Control Positive	Treatment

Figure 1. text