AROGEN: NOVEL ISCHEMIC STROKE THERAPY USING BETA GLUCAN EXTRACT FROM *SACCHAROMYCES CEREVISIAE* ON *RATTUS NORVEGICUS* ISCHEMIC STROKE MODEL, A HISTOPATHOLOGICAL STUDY

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Nur Nadia binti Abdul Halim

Abstract

Stroke is ever increasing in incidence and prevalence and in Indonesia, stroke is the number one cause of death according to a survey conducted by *Kementerian Kesehatan Republik Indonesia*. Current treatment of ischemic stroke by tissue plasminogen activator (r-TPA) targets the removal of clot that occludes the cerebral blood flow causing ischemia. However the use of r-TPA is not without risks and adverse side effects. Studies show that action of r-TPA on a blood clot increases the risk of hemorrhage and neuronal cell death. An alternative form of treatment of stroke is using extract of beta glucan, a glucose polymer obtained from the cell walls of unicellular yeast, *Saccharomyces cerevisiae*. This histopathological study observes the effects of beta glucan on rats (*Rattus norvegicus*) that were induced with ischemic stroke using the middle cerebral artery occlusion (MCAO) method. Rats were divided into 5 groups; negative control group, positive control group and 3 treatment groups. The treatment groups were further divided into beta glucan concentrations of 18 mg/kgBW, 36 mg/kgBW and 72mg/kgBW. The rat brain tissues were extracted and stained using hematoxylin and eosin (HE). The positive control group exhibited hypoxic neurons with nucleus having undergone pyknotic changes. Alterations of the cerebral parenchyma in the form of perivascular edema were also observed. The beta glucan treated groups exhibited lesser pyknotic changes in nucleus and lesser cerebral changes with increasing dosage of beta glucan extract. Histopathological tissue of rats treated with 36 mg/kgBW of beta glucan showed results closest to untreated rats. In conclusion, the beta glucan extract have neuroprotective effects on ischemic stroke rats and have the potential to become an alternative treatment for ischemic stroke.

Key words: Ischemic stroke, Beta glucan, Saccharomyces cerevisiae, Histopathology

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Abstrak

Stroke terus meningkat dalam insiden dan prevalensi dan di Indonesia, stroke adalah penyebab kematian nomor satu menurut survei yang dilakukan oleh Kementerian Kesehatan Republik Indonesia. Pengobatan saat ini dari stroke iskemik oleh aktivator plasminogen jaringan (r-TPA) menargetkan penghapusan bekuan darah yang menyumbat aliran darah otak yang menyebabkan iskemia. Namun penggunaan r-TPA bukan tanpa risiko dan efek samping yang merugikan. Studi menunjukkan bahwa tindakan r-TPA pada gumpalan darah meningkatkan risiko perdarahan dan kematian sel neuron. Bentuk alternatif pengobatan stroke adalah menggunakan ekstrak beta glukan, polimer glukosa yang diperoleh dari dinding sel ragi uniseluler, Saccharomyces cerevisiae. Studi histopatologis ini mengamati efek beta glukan pada tikus (Rattus norvegicus) yang diinduksi dengan stroke iskemik menggunakan metode oklusi arteri serebral tengah (MCAO). Tikus dibagi menjadi 5 kelompok; kelompok kontrol negatif, kelompok kontrol positif dan 3 kelompok perlakuan. Kelompok perlakuan selanjutnya dibagi menjadi konsentrasi beta glukan 18 mg / kgBB, 36 mg / kgBB dan 72mg / kgBB. Jaringan otak tikus diekstraksi dan diwarnai menggunakan hematoxylin dan eosin (HE). Kelompok kontrol positif menunjukkan neuron hipoksia dengan nukleus yang mengalami perubahan pyknotic. Perubahan parenkim serebral dalam bentuk edema perivaskular juga diamati. Kelompok yang diberi beta glukan menunjukkan perubahan pyknotic yang lebih rendah pada nukleus dan perubahan otak yang lebih kecil dengan meningkatnya dosis ekstrak beta glukan. Jaringan histopatologis tikus yang diobati dengan beta glukan 36 mg / kgBB menunjukkan hasil yang paling dekat dengan tikus yang tidak diobati. Kesimpulannya, ekstrak beta glukan memiliki efek neuroprotektif pada tikus stroke iskemik dan berpotensi menjadi pengobatan alternatif untuk stroke iskemik.

Kata kunci: Stroke iskemik, Beta glukan, Saccharomyces cerevisiae, Histopatologi

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LIST OF ABBREVIATIONS

R. norvergicus	*Rattus norvergicus*
S. cerevisiae	*Saccharomyces cerevisiae*
β-glucan	Beta glucan
BPJS	Badan Penyelenggara Jaminan Sosial
FDA	Common Food and Drugs Administration
r-TPA	Recombinant tissue plasminogen activator
NMDA	N-methyl-D-aspartate
BDNF	Brain derived neurotrophic factor
CBF	Cerebral blood flow
ATP	Adenosine triphosphate
AMPA	Alpha-amino- 3-hydroxy-5-methyl-4-isoxazopropionic acid
ROS	Reactive oxygen species
NO	Nitric oxide
AHA/ASA	American Heart Association/American Stroke Association
CREB	CAMP response element binding protein
TTX	Tetradotoxin
β-1,4-glucan	Beta-1,4-glucan
β-1,3-glucan	Beta-1,3-glucan
β-1,6-glucan	Beta-1,6-glucan
mg/kgBW	Milligram per kilogram body weight
cm	Centimeter
%	Percent

CHAPTER 1
INTRODUCTION

1.1 Background

Stroke is ever increasing in incidence and prevalence. Stroke is the top 10 cause of death in United States. In Indonesia alone, stroke is the number 1 cause of death according to a survey conducted by Health Ministry of Republic of Indonesia. To combat stroke, respective government and non-governmental agencies have come up with many preventive and health promotion solutions, yet the number of stroke incidence continue to increase. The cost of stroke varies by stroke type, prognosis and status, however they are generally high. According to the national health insurance (BPJS) 2016, the cost to cover stroke by the national body was around 8% of all catastrophic diseases from the years 2014 to 2016 and amount up to 1.274 billion Indonesian Rupiah. Stroke in Indonesia is highest in South Sulawesi and accounts for 17.9% of all stroke cases in Indonesia. More women are affected by stroke as compared to men. Stroke is an emergency condition whereby the golden period to prevent irreversible complications is an hour as soon as onset of symptoms occur (Saver et al., 2010). Thus to prevent complications from stroke caused by irreversible ischemia, a group of physicians in UK in 1998 created the mnemonic FAST which stands for Facial drooping, Arm weakness, Speech difficulties and Time. Conditions that increases the risk of stroke includes hypertension, high cholesterol, atrial fibrillation and diabetes.

Stroke can be further divided into two; ischemic type stroke and hemorrhagic type stroke. 80% of all strokes are of the ischemic type. Ischemic

type stroke is caused by an emboli, either a thrombotic blood clot or fat that obstructs the blood flow to the specific part of the brain (Durukan and Tatlisumak, 2007). Reduced blood flow causes ischemia of the brain tissues and induces neuronal cell death. Irreversible ischemia induces signs and symptoms that correlate with the location where ischemia has occurred. If ischemia occurs at the motor cortex, the correlating symptom would be hemiparesis or monoparesis and rarely quadriparesis. Signs and symptoms can occur in combination especially when the area of ischemia has spread. Acute stroke medications can reverse complications of stroke if the stroke was managed within 60 minutes from onset. This includes requires quick optimization of cerebral perfusion by attending to patient's airway, breathing and circulation (ABC) and treat hypoglycemia if hypoglycemia is present. If ischemic stroke is present, consider giving the patient thrombolysis or thrombectomy (Kasper et al., 2015).

Treatment of ischemic stroke targets the removal of clot that occludes the cerebral blood flow causing ischemia. Common Food and Drug Administration (FDA) approved drugs include recombinant tissue plasminogen activator (TPA) like Alteplase. r-TPA converts the inactive plasminogen to active plasmin. Plasmin has the ability to lyse long fibrin strands to shorter strands. Breaking down the clot allows blood to continue flowing as normal. This allows quick reperfusion of the brain tissues and preventing it from undergoing further ischemia (Nicole et al., 2001). According to a study conducted by Marler et al, early stroke treatment using tissue plasminogen activator Alteplase reduces the complications of stroke. They defined early treatment at sometime between 90 minutes from the onset of stroke (Marler et al., 2000). However the use of r-TPA is not without risks and adverse side effects. Studies also show the action of r-

TPA on a blood clot increases risk of hemorrhage (Wang et al., 2004). It also induces activation of N-methyl-D-aspartate (NMDA) receptor that activates neuronal cell death (Nicole et al., 2001).

The body has a very strict homeostatic ability. It is able to protect and prevent from degeneration caused by toxic metabolic products. When the NMDA receptors are activated by apoptotic ligand glutamate, cells react by the production of protective neurotrophic protein; brain derived neurotrophic factor (BDNF). BDNF binds to a tyrosine kinase β receptor to trigger a neuronal cascade that will protect the cells from excitotoxicity induced by glutamate-activating NMDA receptor (Lai et al., 2014). While not many scientific publications have been made about the tyrosine kinase β receptor, the potential for the receptor to be made a target for drug development studies look promising as the activation generates a cascade of protective outcome.

A ligand that can increase BDNF levels include the beta glucan extracted from *Saccharomyces cerevisiae*, a unicellular yeast easily cultured in labs. This research will look at the supposed neuroprotective effect of *S. cereviasiae* on rats induced with ischemic stroke.

1.2 Problem Statement

What will be the effect of giving beta glucan extract from *S. cerevisiae* on histopathological analysis of brain tissue of rats induced with ischemic stroke?

1.3 Objective

To observe the potential of beta glucan crude extract from *S. cerevisiae* to reduce the brain damage seen on histopathology of ishemic stroke models.

1.4 Significance

Applicative significance :

To apply the therapy to the consuming market and provide a more affordable alternative to stroke therapy.

CHAPTER 2
LITERATURE REVIEW

2.1 Stroke

Stroke is a neurological disease with an origin in circulation. According to WHO, stroke is defined as the "rapidly developing clinical signs of focal disturbance of cerebral function lasting more than 24 hours or leading to death with no apparent cause other than that of vascular origin" (Conditions, 2008). In 2007, stroke is the 3rd leading cause of death in the US, despite being a preventable and treatable disease. There are 2 types of stroke; ischemic and hemorrhagic stroke. 80% of all strokes are of the ischemic type. Effects of stroke include permanent disability (Hinkle and Guanci, 2007).

The pathophysiology of ischemic stroke is caused by one or several occlusions in the cerebral arterial causing reduced flow (Thus according to Changhong Xing et al the quickest way to reverse this is by reperfusion using rTPA). The occlusion can be caused by either an emboli or a thrombus. The occlusion quickly reduces cerebral blood flow (CBF) and this slows or halts the transport of glucose and oxygen that are the energy sourc es of the neurons. While reduced glucose and oxygen begin activation of neuronal cell death, it does not result in actual death of the neurons yet, and this is called the penumbra. During the state of penumbra, the neurons can be salvaged from death using neuroprotective therapies.

Neuronal death mechanism begins with the reduction in aerobic metabolism and increased anaerobic metabolism, thus increasing lactic acid production and reducing ATP production. The reduced ATP causes an ionic

imbalance and the release of excitotoxic neurotransmitter glutamate. The glutamate binds to the N-methyl-D-aspartate (NMDA) receptor and Alpha-amino-3-hydroxy-5-methyl-4-isoxazopropionic acid (AMPA) receptors. The binding of ligand to receptor causes the opening of calcium channels and an influx of calcium into the neuronal cells. Activation and catabolism by enzymes proteases, lipases and nucleases take place. Glutamate receptors activation also cause influx of sodium and water causing swelling, edema and shrinkage of extracellular spaces. In total, the excess calcium, sodium and ADP increase production of oxidative radicals; reactive oxygen species (ROS) and this brings damage to the cytoplasmic contents of neurons. As a result, neurons die from a combination of cellular death process; necrosis, apoptosis and autophagy (Xing et al., 2012).

The process of inflammation takes place concurrently with neuronal cell death. The most important cell during inflammation is the microglia. During non-pathologic conditions, resting microglias watch over synapses and function in the remodeling and growth and development of neural tissues. Other functions of the microglia include interacting with axons, angiogenesis and phagytosis during apoptosis. At the event of an ischemic attack, microglias proliferate and reach peak proliferation at 48-72 hours post-attack, and would maintain its numbers until several weeks after the initial injury. The microglias are both inflammatory and anti-inflammatory. This occurs due to the ability of the 'resting microglias' to become activated into two different phenotypes; inflammatory phenotype (M1) and anti-inflammatory phenotype (M2). The classically activated pro-inflammatory microglia (M1) produces ROS, nitric oxide (NO) and proinflammatory cytokines TNF-alpha and IL-1. Alternatively activated and anti-inflammatory microglia (M2)

releases anti-inflammatory cytokines and neurotrophic factors GDNF, bFGF, insulin-like growth factor (IGF-1), TGF-beta, VEGF and BDNF (Xing et al., 2012).

The commonly known symptoms of stroke are abbreviated into an acronym; FAST based on the Cincinnati Prehospital Scale. FAST stands for face drooping, arms unable to be raised, speech slurred or jumbled and time. Time indicates the need to call for medical help as soon as possible since the effects of stroke are still reversible within its golden period. The FAST acronym is normally used by non-medical personnel to quickly detect if a patient is undergoing a stroke attack to get the fastest help possible. Medical personnel are advised by the AHA 2018 Guideline to evaluate possible stroke patients using the Stroke Scale by NIHSS which evaluates 13 items (level of consciousness, orientation, response to command, gaze, visual field, facial movement, motor function of both arms and legs, limb ataxia, sensory, language/ aphasia, articulation/ dysarthria, and inattentiveness/ extinction) (Powers et al., 2018).

According to the AHA/ASA 2018 Guideline for Acute Ischemic Stroke, the first-line treatment is tissue plasminogen activator (tPA), alteplase by intravenous line. Alteplase acts to activate plasmin, a protein which would lyse the fibrin clot. However, the treatment using alteplase is only recommended within 3 or 3 to 4.5 hours from within symptom onset or the last known time patient was well. The recommended dose by AHA/ASA are 0.9 mg/kg with a maximum dosage of 90 mg over 60 minutes with 10% of the dose is given as bolus at the 1st minute (Powers et al., 2018). However, the treatment of stroke does with alteplase does not contribute to preserving synaptic plasticity. Thus, once the damage of stroke has been inflicted onto the neurons, it cannot be reversed.

2.2 BDNF

Brain-derived neurotrophic factor (BDNF) is a protein that can be found in both the brain and plasma. In the brain, BDNF originates and synthesized by the neurons while plasma BDNF have an unknown origin (Béjot et al., 2011). The synthesis of BDNF are triggered by activation of NMDA receptor and the glutamate as its ligand. Binding of glutamate to NMDA receptor can be observed in the pathophysiology of ischemic stroke. The process continues with release of calcium and their influx into the neurons to induce neuroexcitotoxicity. CAMP response element binding (CREB) protein are produced and act as a transcription factor which upregulates BDNF production. Other factors that increase BDNF production include reduced neuronal matrix nutrition and the disinhibition of neurons. BDNF would upregulate its own production in a positive feedback loop (Lai et al., 2014). However, BDNF cannot pass through the blood brain barrier (BBB) and must be given via an alternative transport system (Zhang and Pardridge, 2006).

The functions of BDNF are largely discussed in post-ischemic stroke patients due to the detrimental effects low amounts of BDNF have on patients. The two widely discussed effects of BDNF are motor behavior and mental health. BDNF production increases post ischemic stroke as a natural process and plays an important role in post-lesional plasticity. Ploughman et al conducted an experiment to observe the effects of rehabilitation therapy on post ischemic stroke rats and BDNF. The results showed that when the transcription of endogenous BDNF were blocked, rats that were given rehabilitation therapy were not able to recover their motoric functions, similar to rats which were not given rehabilitation therapy (Ploughman et al., 2009). Vaynman et al found a significant

relation between BDNF levels and exercise. Exercising was found to increase calcium influx, which is similar to the glutamate activated excitotoxicity, and thus induce production of CREB. The step down effect of this is the increased production of BDNF (Vaynman et al., 2003). Another experiment conducted by Zhang et al found that BDNF when inserted into the rat brain post ischemic stroke, the rats were able to regain their motoric function (Zhang and Pardridge, 2006). Thus it is proven that BDNF is necessary for the recovery of motoric functions post-ischemic stroke.

Other implication of BDNF in stroke is the correlation between levels of BDNF and post-stroke depression (PSD). Patients with low serum BDNF have shown to have high likelihood of developing PSD (Yang et al., 2011).

2.3 Glutamate

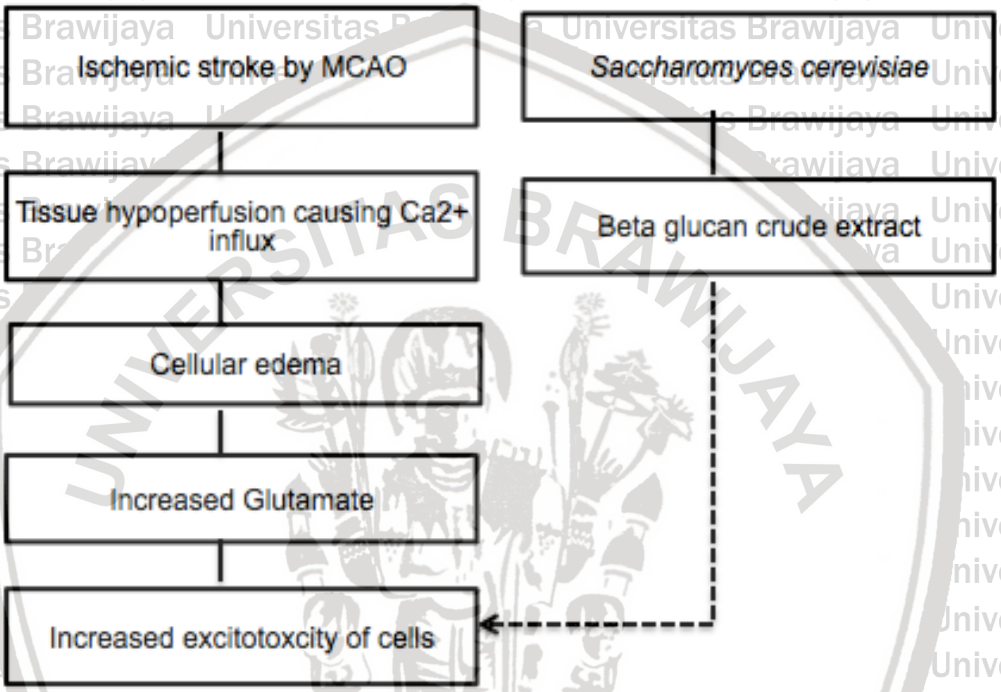
Glutamate is the product of metabolism that plays a key role in causing neuronal death post-ischemic stroke. Glutamate binds to NMDA receptor to cause a cascade of process that will induce calcium ion influx and excitotoxicity. The release of glutamate was found to be inhibited by two processes; the tetrodotoxin (TTX) and magnesium ions. TTX inhibits release of glutamate by inhibiting the voltage gated sodium channels while magnesium ions inhibit the synaptic release of glutamate (Lai et al., 2014).

2.4 Beta glucan

Beta glucan is derived from the cell wall of the unicellular yeast, *Saccharomyces cerevisiae*. It is the component that holds the make up of the cell wall together, preventing the wall disintegration. Beta glucan is composed of carbohydrate polymer chains. The function of the beta glucan is mainly in forming the cell wall of *S. cerevisiae*. The cell wall provides the yeast its rigid structure and prevents the yeast from breaking down due to high internal osmotic pressure. Beta glucan is also found in the cellulose of plants. However the molecular structure of beta glucan in yeast is different to those of plants. Plant beta glucan are composed of the β-1,4-glucan structure while those of yeast are composed of β-1,3-glucan and β-1,6-glucan (Shahinian and Bussey, 2000). Beta glucan have a wide variety of functions. Many researches have proven beta glucan are highly modulating of the immune system and patients with fungal infection exhibit high concentrations of plasma beta glucan with anti-inflammatory cells. Researches have also shown if beta glucan can increase resistance by inducing anti-infective properties to the body. Anticarcinogenic activities have also been proven to be a property of beta glucan and thus can be used as an adjuvant in chemotherapies and radiotherapies (Akramienė et al., 2007). Because beta glucan are found in many food sources, beta glucan are assumed to be safe for oral consumption (Ahmad et al., 2012).

CHAPTER 3
CONCEPTUAL FRAMEWORK AND HYPOTHESIS

3.1 Conceptual framework



Legend:

Inhibitory effect

Activating effect

The rats were induced with ischemic stroke by MCAO method and this would cause reduced blood flow to the brain. Cellular changes take place and cells undergo edema. The cellular edema would cause an increase in the excitatory neurotransmitter glutamate. Glutamate binds to NMDA receptor, thus causing an influx of Ca2+ into the cell. This massive influx would cause cells to undergo apoptosis and this would translate to the changes in phenotype of rat. This was observed by observing the changes in behavior of the rats and if it failed to produce normal movement in the ladder-rung test. Using the *S. cerevisiae* as a treatment method, the crude beta glucan extract was obtained. The crude extract was given to rats at concentrations of 18, 36 and 72 mg/kg BW for 2 months as therapy. After 2 months, the brain tissues were extracted and histopathological analysis were obtained to observed the differences between brain tissues which were induced with ischemic stroke but not treated and brain tissues of rats which were treated with beta glucan crude extract therapy.

3.2 Hypothesis

The *S. cerevisiae* beta glucan crude extract is able to reduce glutamate released and produce less brain damage on histopathological images.

CHAPTER 4
METHODOLOGY

4.1 Study Design

This study used randomized post-test study controlled group design. The objective of this study was to observe changes in behavior in rats (*Rattus norvegicus*) and the histopathology of brain tissues after treatment with beta glucan crude extract from *Saccharomyces cerevisiae*.

4.2 Location and Time of Study

The study was conducted at the Pharmacology and Biomedical Laboratories of Faculty of Medicine Brawijaya University.

4.3 Study Sample

4.3.1 Sample Inclusion and Exclusion Criterias

The study model used were *Rattus norvegicus* male Wistar rats.

1. Inclusion criteria : Gender of rats, age between 7-9 weeks, body weight of 150-170 gram, without any treatment and in healthy conditions observed from movement and fur condition
2. Exclusion criteria : Rats that die during experiment

4.3.2 Estimation of Sample Size

The estimation of sample size were according to the formula below,

P (n-1) > 15 ; P : no. of groups that will be manipulated,

n : no. of repeats

P = 4, thus

4 (n-1) > 15

n – 1 > 15:4, n > 4.75

Therefore this study used 5 amount of samples per groups.

4.4 Study Variables

4.4.1 Independent Variables

Treatment of rats using beta glucan extract from *Saccharomyces cerevisiae* with different concentrations.

1. Group 1: Negative control group (No inducement of stroke and no therapy)
2. Group 2: Positive control group (Inducement of stroke with MCAO and no therapy)
3. Group 3: Inducement of stroke with therapy of crude extract of beta glucan at 18 mg/kg BW
4. Group 4: Inducement of stroke with therapy of crude extract of beta glucan at 36 mg/kg BW
5. Group 5: Inducement of stroke with therapy of crude extract of beta glucan at 72 mg/kg BW

4.4.2 Dependent Variables

Brain tissue changes seen on histopathological study from the brain tissues of negative control, positive control and treated rat groups.

4.5 Operational Definition

- Ischemic stroke model using rats (*Rattus norvegicus*).

  The Wistar male rats were used as an animal model. Inducement of ischemic stroke by MCAO method was to mimic the pathophysiology of ischemic stroke in humans.

- Beta glucan extract from *Saccharomyces cerevisiae*

  Beta glucan crude extract were obtained from liquid culture unicellular yeast *S. cerevisiae* and then used to treat groups of rat models.

4.6 Materials

4.6.1 For Rats

25 plastic cases with measurements of 45 cm x 35.5 cm x 14.5 cm, 25 water bottles, 6 bags, weight scale and pellet for food.

4.6.2 For Stroke Inducement

Xylazine-Ketamine (a double anaesthetic), monofilament nylon sewing thread covered with silicon with length of 40mm.

4.6.3 Therapy of Crude Extract of *Saccharomyces cerevisiae*

Crude extract of *Saccharomyces cerevisiae*, plastic feeding tube.

4.6.4 Surgery

Surgical scissors, pinset, needle pin, Styrofoam, cotton bud, chloroform, alcohol, 25 plastic cases and lids.

4.6.5 Histopathological Study of Brain Tissue

Tissue case, object glass, 70% alcohol, 0.5% gelatin, water, hotplate, incubator, Hematoxylin and eosin staining.

4.7 Study Procedure

4.7.1 Crude Extract of Beta Glucan from *Saccharomyces cerevisiae*

Cultured *S. cerevisiae* were centrifugated and filtered using lysis buffer and repeated 5 times then resuspended at 0 degrees celcius. The cell was lysed with a glass bead Omnimixer for 30 seconds and separated via centrifugation at 3000G for 10minutes. The pellet fraction were obtained by removal of the supernatant. The pellet fraction were cleaned with sterile water and centrifuges at 0 degrees Celcius 3000G for 3 minutes and repeated for 5 times. The pellet was then cleaned using NaCL 5% and centrifugated at 0 degrees Celcius 3000G for 3 minutes and repeated for 5 times. The pellet was then frozen at -40 degrees Celsius.

4.7.2 Ischemic Stroke Induction

The rats were placed on a thermal blanket to control the body heat at 37 degrees Celcius. Ischemic stroke was induced by middle cerebral artery occlusion (MCAO) method, specifically at the right brain. The rats were anaethesized with xylazine ketamine, a double anaesthetic agent and ligated to close the right cerebral artery. Ligation was removed after 120 minutes.

4.7.3 Therapy With Crude Extract Of BetaGlucan

Rats in treated groups were given treatment dosages of 18, 36 and 72 mg/kgBW. The extracts were given via a feeding tube.

4.7.4 Surgery

The rats were placed on anesthesia per inhalation with chloroform. The anesthesized rats were fixated on a Styrofoam and incisions beginning from the stomach. The rat brain tissue from the right brain were obtained.

4.7.5 Histopathological Preparation Of Brain Tissue

Embedding process was carried out by embedding the tissues in liquid paraffin for a couple of hours. The paraffin would solidify with the brain tissue. The object glass is then coated with 70% alcohol for 24 hours, dried and dipped in warm gelatin. Next, the tissues were cut into 4 micrometer width and placed on warm water with 38-40 degrees Celsius. The tissues were picked up using the fixed object glass and dried by placing on a hotplate. The samples were incubated in temperature of 36-40 degrees Celcius for 1 day and then stained with hematoxylin and eosin. The samples were analyzed using magnification of 400X.

4.8 Study Schedule

No	Activity	Month 1				Month 2				Month 3				Month 4			
Preparation		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	Ethical clearance																
2	Laboratory permission																
3	Material acquisition																
4	Rat acclimatization																
implementation																	
1.	Stroke induction																
2.	Therapy of crude extract from *Saccaromyces cerevisae*																
3.	Pembuatan slide histopatologi pada jaringan otak																
Completion																	
	Data analysis																
	Final report writing																

CHAPTER 5
RESULTS

5.1 Qualitative Histopathological Analysis of Positive Control and Negative Control Groups

The histopathology analysis was carried out qualitatively. The changes in the rat neuronal tissues were observed and compared.

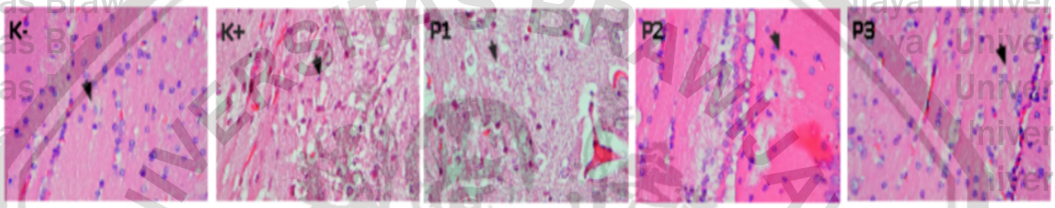


Figure 5.1 Histopathological analysis of rat neurons post-HE staining. (K-) Negative control, (K+) Positive control, (P1) Treatment group 1 at dose 18 mg/kgBW, (P2) Treatment group 2 at dose 36 mg/kgBW, (P3) Treatment group 3 at dose 72 mg/kgBW

Based on the histopathological analysis after staining with hematoxylin and eosin, the control group (K) exhibited normal morphological neurons with intact nucleus. The positive control group (K+) presented hypoxic neurons after middle cerebral artery occlusion. These neurons were observed as hypoxic due to the presence of perivascular edema and reduced sizes of the nucleus, or also termed as pyknosis.

5.2 Qualitative Histopathological Analysis of Treatment Groups

From the experiment, the treatment group 1 (P1) treated with beta glucan extract of 18 mg/kgBW had amounts of neurons exhibiting cells with perivascular edema and pyknosis, which is similar to positive control.

Rat tissues in treatment group 2 (P2) with beta glucan concentration of 36 mg/kgBW exhibited fewer amounts of neurons with pyknosis and perivascular edema.

Rat tissues in treatment group 3 (P3) with beta glucan concentration of 72 mg/kgBW had the least amount of neurons with pyknosis and perivascular edema. When compared with controls, the structures of the neurons were almost similar to normal untreated tissues.

CHAPTER 6

DISCUSSION

From the results obtained, effects of stroke were decreased with increasing dosages of beta glucan crude extract. At 18 mg/kg BW, there was still visible perivascular edema on the neuronal tissues. However, the amounts of neurons with perivascular edema resulted from stroke reduce with increasing beta glucan dosages.

In ischemic rats, the observable changes in histopathological analysis is the perivascular edema and pyknotic changes of the nucleus. A study by Li et al found that rats which were transiently ischemic would produce pyknotic changes in the nucleus, cytoplasmic eosinophilia and necrosis when the ischemic was prolonged (Li et al., 2000). Similar to their study, results observed showed that ischemic rats exhibited pyknotic changes and perivascular edema in the stroke induced rats.

Beta glucan treated rats exhibited a positive progression of protection against the effects of ischemia. The severity of perivascular edema and pyknosis were reduced with increasing dosages of beta glucan. Ischemic stroke induces the formation of reactive oxygen species (ROS). A study conducted by Kayali et al observed beta glucan to have an antioxidant effect. They proposed beta glucan can induce activation of macrophages thus being able to actively uptake ROS and prevent neuronal damage (Kayali et al., 2005).

Ischemia induced rats undergo excitotoxicity by influx of calcium ions. This is the first step to initiating inflammation that will then when prolonged

develop to necrosis of neuronal tissues (Mergenthaler et al., 2004). Beta glucan extracts from other origins like *Candida albicans* have proven to be anti-inflammatory (Du et al., 2015). The histopathological results propose that beta glucan obtained from the *S. cerevisiae* extract may also be anti-inflammatory as it is able to protect rat neurons from undergoing pyknosis and perivascular edema which is the pathological process of stroke that is normally observed.

CHAPTER 7
CLOSING

7.1 Conclusion

In conclusion, beta glucan extracted from *S. cerevisiae* is able to modulate the effects of stroke that is normally seen histopathologically. The dosage that best protects the neurons from the effects of stroke is 72 mg/kgBW for rats. This opens the door to utilizing beta glucan extract as an alternative and cost effective treatment for stroke. Furthermore, the unknown pathways of stroke modulation by beta glucan extracted from *S. cerevisiae* unfold new opportunities into research of that area.

7.2 Suggestions

The research is still lacking in many areas. One of which is the toxicology study for human consumption and the necessary dosage for humans to obtain similar neuroprotective effects as seen in the animal study.

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APPENDICES

Appendix 1

Fourier Transform Infrared Spectroscopy (FITR) Result for Beta Glucan

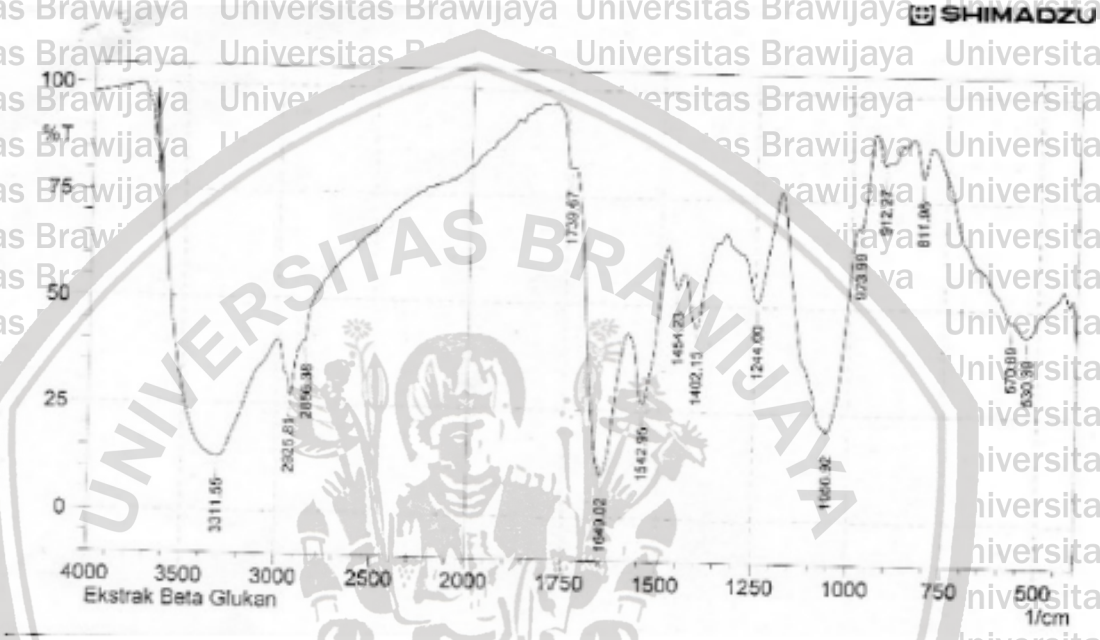


Table Showing the Characteristic of Extract based on Absorption Bands (cm^{-1})

Absorption band (cm^{-1})	Karakterisasi gugus fungsi
3311.55	OH *stretching vibration*
2925.81	CH *stretching vibration*
1649.02	Amide bands
1542.95	Amide bands
1056.92	v(C–C) dan v(C–O–C)
912.27	β-glycosidic bond

Appendix 2

Analysis Report

KEMENTERIAN RISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS BRAWIJAYA FAKULTAS MIPA
JURUSAN KIMIA

Jl. Veteran, Malang 65145, Jawa Timur, Indonesia Telp.: +62-341-575838, fax: +62-341-554403
http://kimia.ub.ac.id; email: kimia@ub.ac.id

LAPORAN HASIL ANALISA

NO : IR.08 / RT.5 / T.1 / R.0 / TT. 150803 / 2016

1. Data Konsumen		
Nama	:	Arinal Mufidah
Instansi	:	Fakultas Kedokteran Universitas Brawijaya
Alamat	:	Jl. Veteran Malang
Telepon	:	082136909798
Status	:	Mahasiswa
Keperluan Analisis	:	Uji Kualitas
2. Sampling Dilakukan Oleh	:	Konsumen
3. Identifikasi Sampel		
Nama Sampel	:	*Ekstrak Beta Glukan*
Wujud	:	Padat
Warna	:	Putih
Bau	:	Tidak Berbau
4. Prosedur Analisis	:	Dilakukan Oleh UPT Instrumentasi Jurusan Kimia FMIPA Universitas Brawijaya Malang
5. Metode Analisis	:	FT-IR
6. Penyampaian Laporan Hasil Analisis	:	Diambil Langsung
7. Tanggal Terima Sampel	:	16 Mei 2016
8. Data Hasil Analisis	:	Terlampir

Catatan:
Hasil analisis ini hanya berlaku untuk sampel yang kami terima dengan kondisi sampel saat itu.

Malang, 17 Mei 2016

Mengetahui:
Ketua Jurusan Kimia,

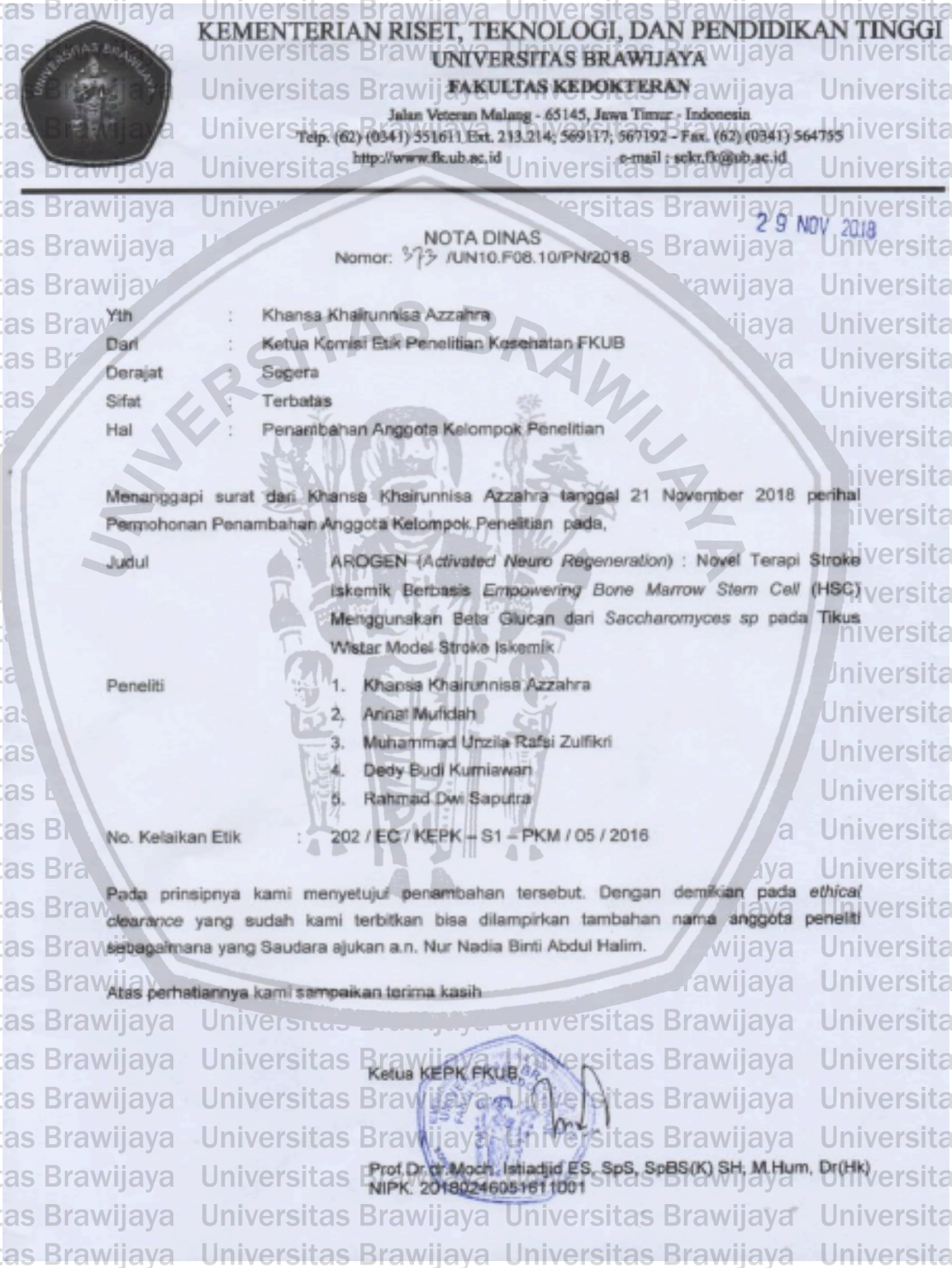
Dr. Edi Priyo Utomo, MS.
NIP. 19571227 198603 1 003

Ketua UPT Layanan Analisa Dan Pengukuran,

Dra. Sri Wardhani, MSi
NIP. 19680226 199203 2 001

Appendix 3

Ethical Clearance



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29 NOV 2018

NOTA DINAS
Nomor: 373 /UN10.F08.10/PN/2018

Yth : Khansa Khairunnisa Azzahra
Dari : Ketua Komisi Etik Penelitian Kesehatan FKUB
Derajat : Segera
Sifat : Terbatas
Hal : Penambahan Anggota Kelompok Penelitian

Menanggapi surat dari Khansa Khairunnisa Azzahra tanggal 21 November 2018 perihal Permohonan Penambahan Anggota Kelompok Penelitian pada,

Judul : AROGEN (*Activated Neuro Regeneration*) : Novel Terapi Stroke Iskemik Berbasis *Empowering Bone Marrow Stem Cell* (HSC) Menggunakan Beta Glucan dari *Saccharomyces sp* pada Tikus Wistar Model Stroke Iskemik

Peneliti :
1. Khansa Khairunnisa Azzahra
2. Annal Mufidah
3. Muhammad Unzila Rafsi Zulfikri
4. Dedy Budi Kurniawan
5. Rahmad Dwi Saputra

No. Kelaikan Etik : 202 / EC / KEPK – S1 – PKM / 05 / 2016

Pada prinsipnya kami menyetujui penambahan tersebut. Dengan demikian pada *ethical clearance* yang sudah kami terbitkan bisa dilampirkan tambahan nama anggota peneliti sebagaimana yang Saudara ajukan a.n. Nur Nadia Binti Abdul Halim.

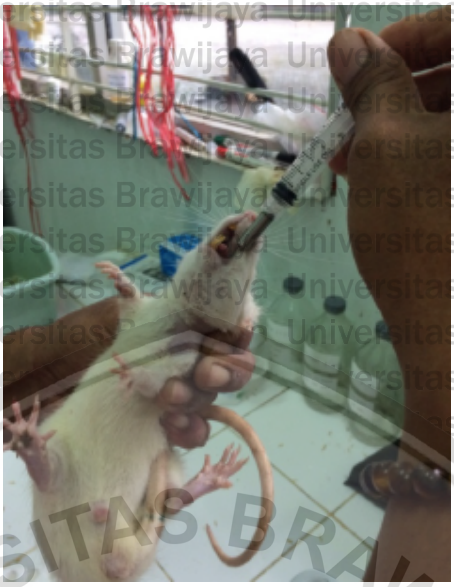
Atas perhatiannya kami sampaikan terima kasih

Ketua KEPK FKUB,

Prof.Dr.dr.Moch. Istiadjid ES, SpS, SpBS(K) SH, M.Hum, Dr(Hk)
NIPK. 2018024605161001

Appendix 4

Documentation



Appendix 5

SK Dekan

Lampiran Keputusan Dekan FKUB
Nomor 339 Tahun 2018
Tanggal 30 NOV 2018

PEMBERIAN PENGHARGAAN KEPADA MAHASISWA BERPRESTASI FAKULTAS KEDOKTERAN UNIVERSITAS BRAWIJAYA PESERTA PIMNAS XXXI DAN ATAU KOMPETISI NASIONAL TINGKAT KEMENTERIAN / DIKTI / LIPI SERTA KOMPETISI INTERNASIONALTAHUN AKADEMIK 2017/2018

NO	NAMA	NIM	KEGIATAN	TINGKAT	CAPAIAN PRESTASI
1	Arinal Mufidah	155070101111096	EXIT MRC FK Unand 2018	Nasional	Juara I Research Paper Congress
2	Mokhamad Fahmi Rizki Sya'ban	155070100111002	EXIT MRC FK Unand 2018	Nasional	Juara I Research Paper Congress
3	Dedy Budi Kurniawan	155070107111042	EXIT MRC FK Unand 2018	Nasional	Juara I Research Paper Congress
4	Nur Nadia Binti Abdul Halim	155070108121004	Intenational Invention & Innovative Competition 2018	Internasional	Gold Medal
5	Muhammad Unzila Rafsi Zulfikri	155070101111090	Intenational Invention & Innovative Competition 2018	Internasional	Gold Medal
6	Agung Dwi Krisnayana	155070101111002	Intenational Invention & Innovative Competition 2018	Internasional	Gold Medal
7	Armareza Putriyani Laili	155070500111001	Bangkok International Intellectual Property, Invention, Innovation and Technology Exposition 2018	Internasional	Gold Medal

8	Ni Putu Ayu Meldayani	155070507111005	Bangkok International Intellectual Property, Invention, Innovation and Technology Exposition 2018	Internasional	Gold Medal
9	Vinta Fajar Ridho Illahi	155070500111025	Bangkok International Intellectual Property, Invention, Innovation and Technology Exposition 2018	Internasional	Gold Medal

DEKAN

SRI ANDARINI

NIP. 195804141987012001

LETTER OF ORIGINALITY

I,

Name : Nur Nadia binti Abdul Halim

NIM : 155070108121004

Study Programme : Medical Degree, Faculty of Medicine, Brawijaya University

Hereby verify that I did this thesis. It is my original work and not based on any form of plagiarism. In the future, if my thesis is proven as the work of others, I am willing to be punished as stated by the rules.

Malang, December 2018

Sincerely,

(Nur Nadia binti Abdul Halim)

155070108121004