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RESEARCH ARTICLE

The Effect of Saccharomyces Cerevisiae on the Serum Creatinine Levels of Chronic Kidney Disease Mice Model

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Abstract

Introduction Chronic kidney disease is pathological condition with various causes, resulting in a progressive loss in kidney function and then ended in end-stage kidney disease. Chronic Kidney Disease become a health problem in the world with increasing number of patients, high progressivity, and bad prognosis. Saccharomyces cerevisiae which has high beta glucan content can increase the level of granulocyte-colony stimulating factor (G-CSF) in the body. The increase of G-CSF level will elevate the excess of hematopoietic stem cell (HSC) in the circulation. The excess HSC can regenerate damaged tubular cells. The study used serum creatinine levels as a marker of improved kidney function. The objective of this research is to determine the effect of S. cerevisae to the serum creatinine levels of mice model with chronic kidney disease and to determine the correlation between dose of S. cerevisae extract and serum creatinin levels of chronic kidney disease mice model. Materials & Methods This in vivo experimental study used a randomized post-test only controlled group design. The samples were divided into five groups, each group consists of 5 mice, that were negative control group, positive control group, Treatment Group 1 (Aristolochic Acid + S. cerevisae extract 50 mg/kg), Treatment Group 2 (Aristolochic Acid + S. cerevisae extract 100 mg/kg), Treatment Group 3 (Aristolochic Acid + S. cerevisae extract 200 mg/kg). The induction of chronic kidney disease were using aristolochic acid 2,5 mg/KgBW 5 times a week for 2 weeks. The variables measured in this research is the serum creatinine levels. Results Statistical data obtained shows that the average number of serum creatinine levels at negative control group was 0,3788 mg/dl in the positive control was 0,6425 mg/dl; in the treatment 1 was 0,4575 mg/dl; in the treatment 2 was 0,3850 mg/dl; in the treatment 3 was 0,3263 mg/dl. ANOVA test results showed significant difference between groups ($p < 0.05$). Conclusions S. cerevisae was able to decrease the serum creatinine levels of chronic kidney disease mice model and there were correlation between increased dose of S. cerevisae and decreased in serum creatinine levels.

Keywords: *Beta Glucan, Saccharomyces cerevisae, Serum creatinine levels, Chronic Kidney Disease.*

Introduction

Chronic kidney disease is one of many health problems occurred in developed or established countries.[1] Chronic kidney disease pathology depend on the underlying diseases such as diabetes mellitus, hypertension, glomerulonephritis, and urinary tract obstruction that will lead to progessive kidney failure.

Progressive damage to the kidney cause inflammatory response, therefore it also increases the number of pro inflammatory cytokine. Inflammatory response also produces Reactive Oxygen Species (ROS) due to cellular oxygenizing stress. These cytokines along with ROS can lead to irreversible nephron damage, inducing glomerular adaptive hyperventilation followed by increasing of glomerular permeability and activation of renin angiotensin aldosterone system (RAAS). These process induce inflammatory nephrotoxic in the kidney that can lead to tubulointerstitial fibrosis. Tubulointerstitial fibrosis is the last pathway leading to chronic kidney disease. Chronic kidney disease can cause decrease in physiological kidney function shown by decrease of GFR (glomerular filtration rate)[2,3]

One of physiological function of kidney is to eliminate most of metabolic waste in our

body (metabolic waste and medicine). Creatinine is metabolic waste product as the result of protein metabolism in the muscle that has to be eliminated [4]. Creatinine is very important in clinical examination because it is the end product of kidney metabolic and couldn't be converted into anything else. Serum creatinine concentration and blood urea nitrogen can be used as parameter for glomerular infiltration rate.

Serum creatinine is a strong and specific indicator of kidney function, increasing of Serum creatinine by two fold indicate decreasing of kidney function as much as 50%. Serum creatinine ranges around 0, 7-1,5 mg/dl. Many factors contributing to sensitivity of creatinine in kidney function test including: accuracy in measuring urine production over 24 hours, muscle mass towards endogen creatinine production, consumption of red meat, physical activity, creatinine secretion in tubular of kidney, and also consumption of medicine [5].

Recently regenerative medication based on stem cell developed rapidly and appoint to developed widely with good medication potential especially to degenerative disease. Stem cell is an underdeveloped cell with the potential to grow into many specific type of cell and developed into embryonic tissue and mature tissue. One of the stem cell type is hematopoietic stem cell (HSC) that came from bone marrow [6]. HCS or hematopoietic stem cell has multipotent characteristics proven in many studies to be able to change into many cells in our body [7].

It can be mobilized from the bone marrow to the damaged kidney, so it can be regenerate. This method has many advantages beside repairing the damaged kidney it is also safe, so risk of injury to the patient can be minimalized during therapy [8]. Mobilization of HSC to the injured tissue induced repair mechanism to the injured kidney tissues cause by chronic kidney disease [9].

One of many factors arised from the release of HSC from the bone marrow to the bloodstream is G-CSF (granulocyte colony stimulating factor). G-CSF increased HSC mobilization process to the bloodstream by releasing bondage of CXCL-12 and CXCR-4, therefore releasing HSC to the bloodstream followed by gradient of chemoattractant CXCL-12 inside the damage tissues, mobilized HSC through the damaged tissue induced tissue repair mechanism, especially damaged kidney tissue caused by chronic kidney disease [9].

Study by Lin et al., on hemotoxicity animal model (2009) proof one way to increased G-CSF (granulocyte colony stimulating factor) is by giving beta glucan. Beta glucan is a polysaccharide contain in Saccharomyces cerevisiae yeast. The yeast is widely use in bread making. Saccharomyces cerevisiae has the most beta glucan account for 60% compared to yeast yield from fungus and other wheat [10].

Based on the previous explanation, it is suspected that Saccharomyces cerevisiae are able to increase secretion of G-SCF to repair the damaged kidney tissue by mobilizing HSC (hematopoietic stem cell) to the kidney. One of the indicator to evaluate improvement of kidney function was serum creatinine. Therefore research to find out the effect of Saccharomyces cerevisae towards serum creatinine in mice model with chronic kidney disease is needed.

Subjects and Methods

This study is a true experimental laboratory study using Randomized Post Test Controlled Only Group Design, with random group sampling. Intraperitoneal induction of aristolochic acid (AA) aimed to create aristolochic acid nephropathy (AAN) in order to make chronic kidney disease model, AA was given 2,5 mg/kg for five days in a week for two weeks (Sato et al, 2004). Doses for beta glucan extract from Saccharomyces cerevisiae is based on fat metabolic of mice atherogenic diet model by Robak et al.

Mice model were divided into five groups consist of, negative control: healthy mice (without induction of Aristolichic Acid and without S. cerevisae extract); positive control (mice was induced with Aristolichic Acid; Group with treatment 1 (A): mice was induce with Aristolichic Acid and given S. Cerevisae extract for 50mg/Kg body weight; 2 (B): mice was induce with Aristolichic Acid and given S. Cerevisae extract for 100mg/Kg body weight; 3 (C): mice was induce with Aristolichic Acid and given S. Cerevisae extract for 200mg/Kg body weight.

Preparation of experimental animal was done by maintenance of cage, knitted wire, hay stack, drinking bottle, spray, food bowl, food, 70% alcohol, experimental animal Babl/C type mice (male, age 6-8 weeks, weighing 20-25 grams). The mice were adapted in parasitology laboratory for 7 days and were divided into 5 groups.

Induction of mice model into chronic kidney disease were using 25mg/Kg body weight with AA diluted in corn oil. Aristolochic acid mechanism is inducing damage to the kidney blood vessels causing ischemia and interstitial fibrosis [11]. Long term induction causing chronic necrosis. First of all, the mice were conditioned to have chronic kidney disease with induction of AA every day for 5 days for 2 weeks through intraperitoneal access [12].

Sachharmonyces cerevisiae yeast, mauripan brand, were crushed with pestle and mortar and weigh for about 100 grams and then placed into beaker glass and diluted in 900ml ethanol 95%. The solution was macerated for 3 days. Evaporator was placed in a permanent pole so it can be hang over the 30°-40° angle toward the table. Macerated solution was placed into extraction flask. One set of evaporation flask base was placed in a specific manner so some part of the extraction flask was soaked in water bath. Water bath was connected to power source and temperature was rised to 70° C (ethanol boiling point).

Wait for the process to work (evaporation and separation in cooling process) until evaporation solute was left in extraction flask. Evaporation solute was a thick solution weighing approximately 60 grams. The extract was diluted using aquadest adjusted to the need of drinking water for every mice group with comparison of (1ml aquadest : 12,5mg extract) then the solution was given to the mice for 2 weeks by feeding tube every afternoon.

Before dissection, anesthesia was given to comfort the mice. Anesthesia used in this study was chloroform placed in a sealed container and inhaled. Mice that has been anesthetized was placed on a styrofoam, fixed and dissection started from the abdomen. Afterward blood was obtained from the heart with 1 ml syringe.

The kidney was taken out and then placed in a container containing PBS (Phosphate Buffer Saline).

Mice kidneys obtained from the dissection was made into histopathology slide to be examine using paraffin method. Fixation was done using 10% formalin over 24 hours then rinsed with water approximately 1, 5 hours. The tissue was placed in 70% alcohol solution for 1 hour, 80% alcohol for 1 hour, 99% alcohol for 1 hour and absolute alcohol for 2 x 1 hour, continued by mixing it with xylol solution: absolute alcohol with comparison of 1:1 for half an hour and xylol PA for 2 x 30 minutes.

Tissue was cut as thin as possible and placed into the melted paraffin: xylen (1:1) for 1 hour, paraffin (54-58) for 2 x 1 hour. Melted paraffin then molded into block, then set to cool. Tissue inside the paraffin block then cut using microtome then placed it into observational glass that are previously covered with mixture of egg white : glycerol (1:1) let it set and dry. Place the observation glass into xylol for 3 x 5 minutes and let it dry.

Observation was made on the cortex of the kidney using 400x power. Blood sample was left over for 15 minutes then centrifuge for 15 minutes with 3000 rpm speed. Clear part (serum) was separated and taken for examination of creatinine. Serum was taken using pipette for 50 ul then place it into experimental flask, mixed with working reagent containing 4 parts of reagent 1 and 1 part of reagent 2 then stir with vortex and absorbance was measured using spectrophotometry. Results of increasing in serum creatinine, control and also treatment group were statistically analyzed using SPSS 16.0 with significant level of 0,05 ($p = 0,05$).

Results

Balb/C mice were induced with AA with 2,5 mg/kg dose for 5 days/week for 2 weeks, with 1 week progressive phase before given *S. cerevisiae* extract. Histopathology result revealed mild fibrosis in the interstitial tissue shown by black arrow and tubular necrosis is shown by the blue arrow. In the negative control group without induction of AA shows normal histopathology of the kidney.

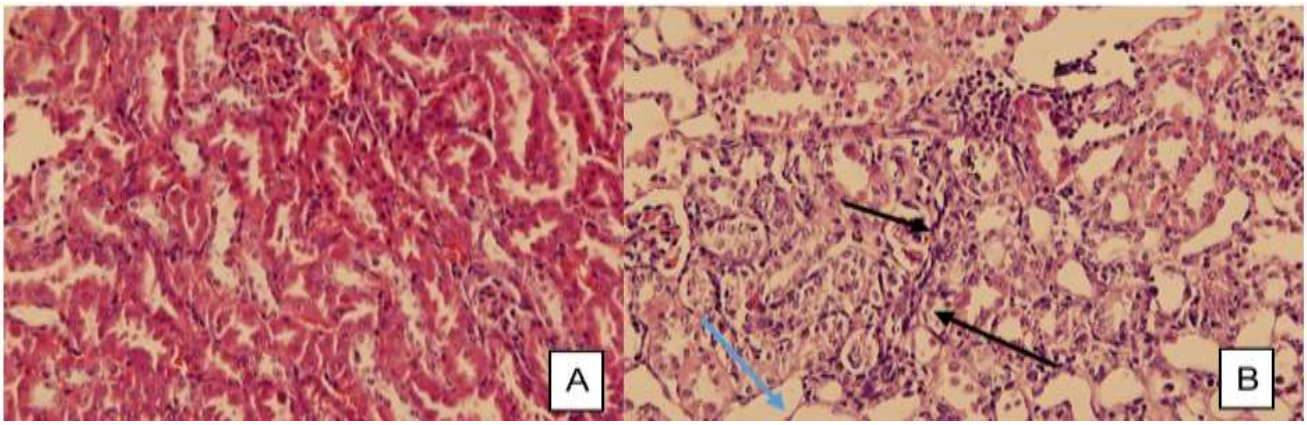

Figure 1: Hematoxylin Eosin coloring from BALB/C chronic kidney disease mice model. Mice was induced to Aristolochic Acid with 2,5mg/Kg dosis every day for 5 days, for 2 weeks with 1 week of disease progression. Black arrow appoint mild fibrosis and blue arrow appoint tubular damage. (A) negative control showing normal kidney, (B) positive control showing interstitial fibrosis and tubular damage in BALB/C mice kidney.

According to this study, serum creatinine was arranged in the table below:

Table 1: Mean serum creatinine

Group	Mean Serum Creatinine
Negative Control	0,3788 ± 0,0983
Positive Control	0,6425 ± 0,1668
Treatment 1	0,4575 ± 0,0602
Treatment 2	0,3850 ± 0,0925
Treatment 3	0,3263 ± 0,0558

Negative control = no AA induction and not given *S.cerevisiae* extract; Positive control = only AA induction; Treatment 1 = *S.cerevisiae* extract 50mg/Kg; Treatment 2 = *S.cerevisiae* extract 100mg/Kg; Treatment 3 = *S.cerevisiae* extract 200mg/Kg

Base on Table 1, we can see that mean serum creatinine in negative control group is 0,3788, while in positive control group is 0,6425, Treatment 1 group = 0.4575, Treatment 2 group = 0,3850 and Treatment 3 group = 0,3263.

From this data it is shown that serum creatinine was the highest in the positive group which is 0, 6425. Base on this data, comparison diagram was shown comparing mean of negative control group, positive control group and treatment group.

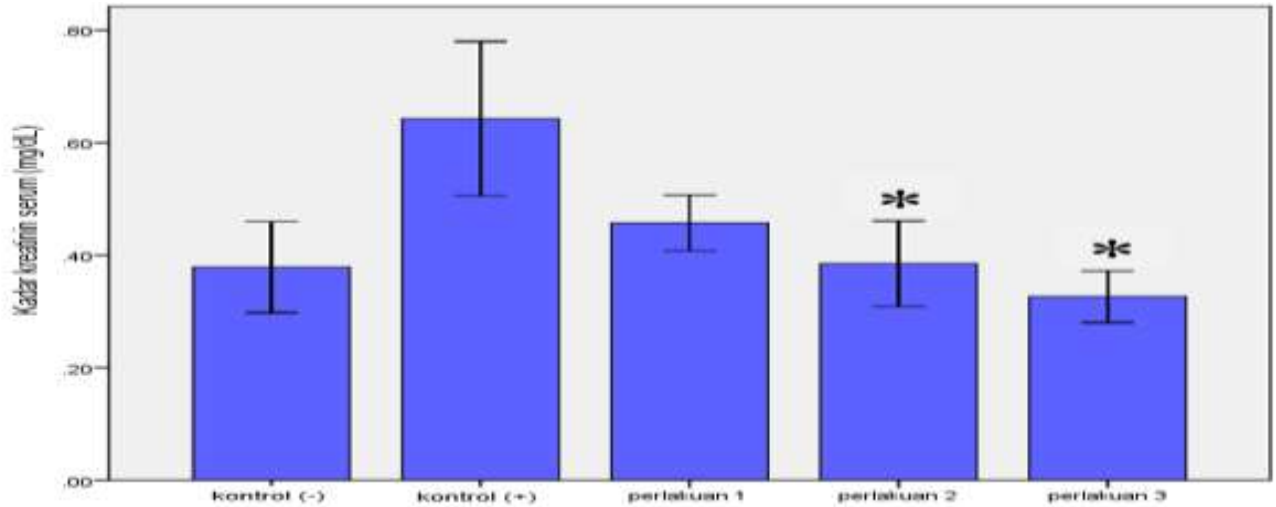

Figure 2: *Saccharomyces cerevisae* effect towords serum creatinine in chronic kidney disease mice model

From the chart, serum creatinine increase significantly in positive control group compared to negative control group. Treatment 1 group (*S.cerevisiae* extract 50mg/Kg), Treatment 2 group (*S.cerevisiae* extract 100mg/Kg) and Treatment 3 group (*S.cerevisiae* extract 200mg/Kg), serum creatinine decreases compared to positive control group. There is also significant decrease in serum creatinine in treatment

group 2 and 3 compared to positive control. *) = significant p<0,05. The data analyzed were result of serum creatinine in chronic kidney disease mice model using SPSS for windows 18.0 with significant of 0,05 (p = 0,05) with 95% confidence interval (α = 0,05). Hypothesis testing used comparative and correlative test started with normality test using (Kolmogorov-Smirnov) and homogeneity test using (Levene). If data distribution was in a normal range, One-Way ANOVA and Post-Hoc test were used to determine different value among groups.

Normality test using Kolmogorov-Smirnov shows normal distribution of data with p = 0,958 (p > 0,05). Meanwhile homogeneity test using Levene, results of homogenous data with p = 0,257 (p > 0,05). Next step is One-Way ANOVA test because the data was in normal distribution and already homogenous with the result of p = 0,005 (p < 0,05). The result shows at least 2 different group were significantly different. Post-Hoc test is needed to find out different value of each group, that can be seen in the Table below:

Table 2: Post-Hoc test

Comparison Between Group		*p-value*	Additional Info
C (-)	C(+)	0,018	Significant
	T1	0,812	Not Significant
	T2	1,000	Not Significant
	T3	0,948	Not Significant
C (+)	T1	0,132	Not Significant
	T2	0,021	Significant
	T3	0,004	Significant
T1	T2	0,852	Not Significant
	T3	0,406	Not Significant
T2	T3	0,924	Not Significant

Post-Hoc test shows significant different in serum creatinine between negative control group and positive control group (p = 0,018). Meaning, induction into chronic kidney disease in mice model, significantly increases serum creatinine level in chronic kidney disease mice model compared to negative control group without induction of Aristolochic Acid. *Saccharomyces cerevisae* treatment group 2 and 3 100mg/Kg, 200mg/Kg) respectively caused significant different in serum creatinine compared to positive control group (*p* = 0,021; 0,004).

The test is continued using Pearson correlation test to proof correlation between increasing of Saccharomyces cerevisae doses to serum creatinine level and the result were negative correlation, meaning the higher the dose of Saccharomyces cerevisae, the lowest the level of serum creatinine in chronic kidney disease mice model.

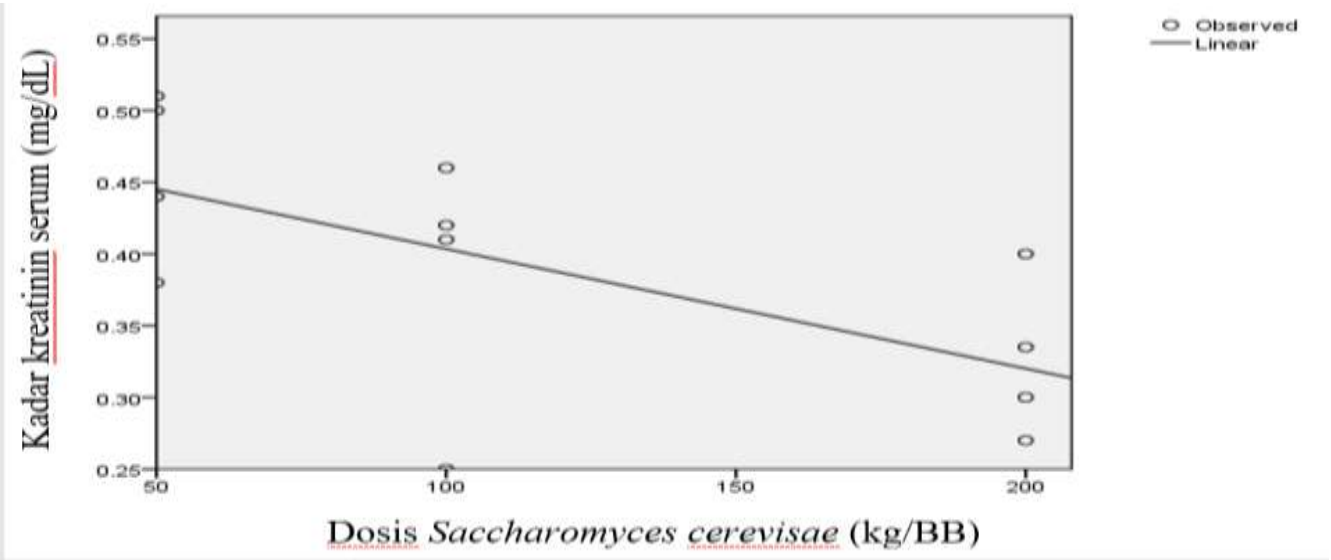

Figure 3: Regression test between increasing of *Saccharomyces cerevisae* doses towards increasing of serum creatinine

From regression test between increasing of dose with decreasing serum creatinine level, the result was $p < 0,05$. Meaning there were correlation between increasing of dose with decreasing of serum creatinine whereas the power of this correlation was strong ($R = -0,635$), this was proven that increasing dose of *Saccharomyces cerevisae* decreases serum creatinine level.

Discussion

This study aimed to find out the effect of *Saccharomyces cerevisiae* towards serum creatinine level in chronic kidney disease mice model. Intraperitoneal induction of aristolochic acid (AA) aimed to induce aristolochic acid nephropathy (AAN) to the mice model turning it into chronic kidney disease [12]

In chronic kidney disease, kidney tissue went through atrophic state, whereas the number of cell decreases. In histopathologic manner it is shown by fibrosis process in tubulointerstitial [13]. Chronic kidney disease induced by aristolohic acid can caused Aristolochic acid nephropathy (AAN), an interstitial progressive nephritis that turns rapidly into chronic kidney disease. As a result, damaged was made in proximal tubular epithelial leading to apoptosis and causing tubular atrophy ending it into chronic kidney disease [14].

Pathophysiology of AAN has major lesion located in the cortex shown by wide interstitial fibrosis with tubular atrophy. Cellular infiltration in the interstitial is very rare. Thickening of interlobular wall and afferent arterioles causing swelling of endothelial cell. Glomerulus didn't swell and deposit of cellular immune was unclear. This condition shows that primary lesion occurred in the endothelial wall of blood vessel causing ischemia and intersitital fibrosis[11]. In vitro and in vivo study induction of aristolochic acid can lead to apoptosis from proximal tubular started by atrophy process of proximal tubular [15].

Aristolohic acid used was proven to induce chronic kidney disease in the previous experiment. Aristolohic acid effect on male mice has higher impact compared to female mice. This is due to hidroxiprolin substance that were greater in male mice by 4,7 times. Hidroxiprolin is a collagen like substance in the kidney, whereas the greater the level, it will worsen the process of interstitial fibrosis [16].

In this study, *Saccharomyces cerevisiae* with the dose of 100 mg/Kg and 200 mg/Kg respectively revealed, decrease in serum creatinine in chronic kidney disease mice model significantly 0,05 ($p = 0,05$) with confidence interval of 95% ($\alpha = 0,05$). Decrease in serum creatinine occurred because of improvement of kidney tissue influence by beta glucan contain in *Saccharomyces cerevisiae*, especially beta 1,3d-glucan [17]. Beta glucan influenced increasing of granulocyte and mobilization of granulocyte and also it's progenitor by stimulation of G-CSF production by macrophage, endothelial cell, and lymphocyte [8,18].

Increasing of GCSF in bone marrow cause neutrophil protease (serine protease) to accumulate in bone marrow. Serine protease lead to regulation of chemokine stromal cell-derived factor-1 (SDF-1 also called CXCL-12) by decreasing of FOXC-1 inside CXCL-12 abundant reticular (CAR) causing relase of CXCR-4 and CXCL-12 bondage [19,20]. Release of ligand receptor bondage lead to mobilization of HSC in the peripheral blood stream. CXCL12 gradient in bone marrow induce strong bondage between HSC circulating in the endothel through CXCR4, followed by mobilization and homing to stromal cell. Hypoxic area inside the bone marrow micro environment shows high CXCL12 concentration therefore it will maintain HSC level. CXCL12 concentration inverse with partial oxygen pressure (pO2).

The lowest the partial oxygen pressure, the highest the concentration of CXCL12, vise versa. Homing from HSC and non-HSC require CXCR4. Hypoxia-inducible factor-1 (HIF-1) induce expression of CXCL12 straight away based on partial oxygen pressure in those area. CXCL12 will recruit any cell expressing CXCR4 in the circulation making it into "conditional" stem cell so it is able to repaired the damaged tissues [21]. Chronic Kidney disease caused by AA will lead to lesion located in center of the vessel leading it to ischemia [11]. Ischemia cause HIF-1to induce expression of CXCL12 causing higher gradient. HSC from circulation will follow the gradient concentration of CXCL-12 from lowest

gradient (bone marrow) to the highest gradient (kidney) [22] HSC mobilization through the kidney can induced repair mechanism, especially kidney cell with chronic kidney disease [9]. Hematopoietic stem cell can altered into many type of mature cell [6]. In chronic kidney disease induce by AA, proximal tubular epithelial damage through apoptosis leading to tubular atrophy and ended as chronic kidney disease [14]. HSC ability to change into mature cell, one of them came from tubular epithelial of the kidney, therefore mobilization of HSC into the kidney is required for healing process of chronic kidney disease [23].

This is also supported by other study, proofing that HSC are able to repair kidney from other study using experimental animals model with ischemic/reperfusion (I/R) injury, glomerulonephritis, and alport syndrome [24-26]. Those studies revealed that hematopoietic stem cell has a role in reparation process of glomerular endothelial cell and mesangial cell.

Physiologic improvement of kidney were marked by decreasing of serum creatinine cause by improvement or regeneration of kidney epithelial cell by HSC. So it can be concluded that *Saccharomyces cerevisiae* extract containing beta glucan are able to decrease serum creatinine level in chronic kidney disease mice model.

Although, this study still has limitation including, this study aimed just to find out the effect of *Saccharomyces cerevisiae* toward decreasing of serum creatinine in chronic kidney disease mice model, meanwhile effective dose of *Saccharomyces cerevisiae* extract to decreased the level of serum creatinine remain unknown. Besides that, side effect from consuming *Saccharomyces cerevisiae* continuously is still unclear.

Conclusion

Saccharomyces cerevisiae are able to decreased serum creatinine significantly in mice model with chronic kidney disease and there were correlation between increasing of *Saccharomyces cerevisae* doses with decreasing of serum creatinine in mice model with chronic kidney disease.

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