THE DYNAMICS OF 1.3-β-D-GLUCAN GIVEN IN THE FORM OF AGARICUS BLAZEI MURILL POWDER TO THE BODY OF WHITE MICE (*Sprague Dawley*) AND EFFECT OF RENAL AND HEPATIC HISTOPATHOLOGY

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ABSTRACT

The dynamics of 1.3-β-D-glucan in the form of ABM powder and the histopathological effect on kidney and liver were conducted to 24 male Wistar rats which were all 2 months old. The experiments were conducted randomly and were divided into 3 different treatment groups: control, ABM powder, and pure 1.3-β-D-glucan compound. All the experiments were completed within 28 days. All the treatments were given in the morning at the 1, 3, 8, 12, and 24 hour periods after data of blood treatment was collected, while the urine and feces was collected every 8 hours. The giving treatment was continued on and on until the 28 day period was completed. This study found that the dynamics of 1.3-β-D-glucan rate in the form of god's mushroom powder inside the body of the rats on the blood samples collected at the 1, 3, 8, 12, and 24 hour after treatment periods were different with that from the control group and provide a certain rate which is close to the pure 1.3-β-D-glucan compound group. Moreover, the effect of the histopathology on the kidneys and livers showed that the amount of cells which experienced necrosis were more than that in the group being given the pure 1.3-β-D-glucan compound group than that in the group being given 1.3-β-D-glucan in the form of ABM powder, both in the kidneys and the livers. This research showed that the use of the pure compound should be handled with more care than that of the 1.3-β-D-glucan in the form of ABM powder in term of the dosage used.

Keywords: Agaricus Blazei Murill, Dynamics, Histopathology.

INTRODUCTION

Agaricus blazei Murill or ABM is a kind of mushroom that can be used as medication. Based on some reearch conducted by Bruggemann *et al* (2006), it was concluded that this kind of mushroom con-tains of 40-45% protein, 38-45% carbohydrate, 6-8% fiber, and the rest 3-4% are fat, vitamin B1, B2, and niacin. According to Naso *et al* (2010), ABM contains polysaccharide compound: 1.3-β-D-Glucan and 1.6-β-D-Glucan. It also contains various kinds of minerals such as iron, potassium, phosphor, magnesium, copper, selenium, manganese, and zinc (Novaes *et al*., 2007). According to Al-DBass *et al.* (2012) and Andrea *et al.* (2013) the mush-room also contains ascorbic acid and tocopherol (vitamin E), agaritine (Rhoupas *et al,* 2010; Akiyama *et al.,* 2011; Nagaoka *et al*., 2006). The chemical ingredients on the god's mushroom, under each singular component may function as an anticancer, are 1.3-β-D-Glucan and 1.6-β-D-Glucan, ergosterol, and agaritine. These natural substances contain an active substance either in the form of its singular or plural compound, depending upon the characteristic of the active substance itself. The natural compound found in the extract, can provide effects that come from the interaction among the components (Heinrich, *et al*., 2010) and give physiologic effects (Spinella, 2002, Xiang, et al., 2011).

Most previous research conducted hitherto merely investigated the active substances in a single component without considering the complexity of each component of the active substances. The activity of complexity of plant has not been focused much in research not discussed much in literature. Therefore, this research aims to look at the dynamics of the active compound of 1.3-β-D-Glucan given in the form of ABM powder and 1.3-β-D-Glucan pure compound in the body of a white rat in order to see the level of 1.3-β-D-Glucan in the blood, urine, and feces. The dynamics meant is the levels of 1.3-β-D-Glucan in the blood samples of 1 hour, 3 hours, 8 hours, 12 hours, and 24 hours after treatment (of the control, ABM powder, and 1.3-β-D-Glucan pure compound group), the levels of 1.3-β-D-Glucan in urine and feces samples of 8 hours, 16 hours, and 24 hours after treatment. With regard to toxicity effect, a histopathology study of kidney and liver is conducted by observing cells that have experienced necrosis.

MATERIALS AND METHOD

ABM Powder

The ABM powder used in this research is the dried god's mushroom which has been made into powder, obtained from an Agaricus traditional industry Sido Makmur Lawang in Malang. The sample used is under a certain amount (7.2 mg).

Test Animal

The animals used for experimentation in this research were Sprague Dawley Rats aged 6 to 7 weeks weighing 200 grams obtained from the D'Wistar unit in Bandung. The number of rats used for determining the levels of the active substance was 9: 3 rats not being given anything as the control group, 3 rats being given 7.2 mg of ABM powder, and 3 other rats being given 1.3-β-D-Glucan 1 mg of pure compound. The rats were kept in metabolic cages equipped with urine and feces container.

Research Procedure

The animal blood sampling was performed to the ABM powder group after 1 hour, 3 hours, 8 hours, 12 hours, and 24 hours being given the ABM powder (Takizawa, Y., et al., 2003; Changuang *et al.*, 2003; Khalid et al., 2009). The blood was taken from the rats' tail, put into eppendorf tubes, was immediately centrifuged at 3,000 rpm speed for 15 minutes to obtain the serum. The serum was then given 1% HCl methanol solution with a ratio of 1:1, and then stored at -20°C container until further analysis was conducted. According to Canguang *et al.* (2013), urine sampling is appropriately completed in 8 hours, 16 hours, and 48 hours after being given the ABM powder, pure compound, and control. The amount of urine used was as much as 1 ml and was immediately added 1% HCl methanol at a ratio of 1:1 prior to sample preparation. This was applied also for feces sampling.

Feces sampling preparation for HPLC analysis

A total of 0.5 g feces sample was dried using an oven at the temperature of 40 to 50 °C until the moisture level reached below 12% which was then crushed using a blender and sieved with sieve mesh 40. The sample was then dissolved in 250ml of aqua bidest and shaken using a shaker for 1 hour. The solution was then filtered in order to obtain the filtrate. The filtrate was then centrifuged at 3000 rpm speed for 10 minutes to obtain supernatant. The supernatant obtained from this process was then filtered again with a filter tool of 0.45 μm polytetrafluoroethylene (Alltech associates, Deerfield, IL) so that it could be continued to HPLC analysis.

Urine sampling preparation for HPLC analysis

A total of 1 ml urine sample was dissolved in 10 ml of aqua bidest and was steered using a vortex for 10 minutes. The solution was then filtered in order to obtain its filtrate. The obtained filtrate was then centrifuged at 3,000 rpm speed for 10 minutes to obtain supernatant. The supernatant obtained from this process was then filtered again with a filter tool of 0.45 μm polytetrafluoroethylene (Alltech associates, Deerfield, IL) so that it could be continued to HPLC analysis.

Serum sampling preparation for HLPC analysis

As much as 50 μl blood sample was dissolved in 1 ml of aqua bidest and was steered using a vortex for 10 minutes. The solution was then filtered in order to obtain its filtrate. The obtained filtrate was then centrifuged at 3,000 rpm speed for 10 minutes to obtain supernatant. The supernatant obtained from this process was then filtered again with a filter tool of 0.45 μm polytetrafluoroethylene (Alltech associates, Deerfield, IL) so that it could be continued to HPLC analysis.

Histopathologic specimen preparation

Paraffin sections of the samples' kidneys/hepars were prepared following the standard procedure (Cui *et al.*, 2009). The kidneys/hepars were then fixed using 10% neutral buffered formalin. After 24 hours, the kidneys /hepars were removed from formalin and stored in 70% ethanol, followed by embedding in parafin, sectioning at 5 microns, and staining with hematoxylin and eosin (HE). Histopathological changes were observed under conventional optical microscope (OLYMPUSBX 41, Japan) and dyno eye camera. For histopathologic assessment, transverse sections were taken through necrosis cell.

Statistical Analysis

The differences of necrosis cell structure were analyzed by One-way analysis of variance (ANOVA) followed by LSD post-hoc test analysis used to pairwise compare differences between different groups using SPSS 22 for windows. P-values < 0.05 were considered to be significant.

RESULTS

Levels of 1.3-β-glucan in serum, feces, and urine samples

The levels of 1.3-β-glucan on the serum of treatments using ABM powder were lower than that of treatments using the 1.3-β-glucan pure compound after 1 hour, 3 hours, 8 hours, 12 hours, and 24 hours of administering the treatment (Figure 1)

Histopathology of Liver and Kidney

Based on the calculation above image obtained cells undergoing necrosis. The strenght of calculation is the average of 10 observations viewing area. The results of the Calculations cis described in Fugure 2 and 3 below.

DISCUSSION

The concentration of 1.3-β-D-Glucan in each serum sample, throughout the time, has experienced concentration addition. Comparison between control, ABM powder, and 1.3-β-D-glucan pure compound in the serum after 1 hour, 3 hours, 8 hours, 12 hours, and 24 hours of

treatment showed significant differences between treatments. The highest concentration of 1.3-β-D-Glucan of the three treatments is in the 1.3-β-D-glucan pure compound treatment. The concentration of 1.3-β-D-Glucan pure compound in the time span of 12 hours has not shown any concentration decline, while that of ABM powder has decreased. This indicates that the 1.3-β-D-Glucan pure compound is easily absorbed in the digestive process than the 1.3-β-glucan found in the ABM powder. According to William *et al* (2011), as long as it is attached to the fungal cell wall the structure of 1.3-β-glucan does not dissolve, but when in the blood or body fluid the structure of 1.3-β-Glucan turns into a singular helix, triple helix, or coil shape to help it dissolve. Based on this, the level of 1.3-β-glucans in ABM powder needs some time to change its structure in order to escape from the fungal cell wall then to become 1,3-β-glucan compound in blood, beside that it should be noted that the powder contains many components, not only the 1.3-β-D-glucan compound.

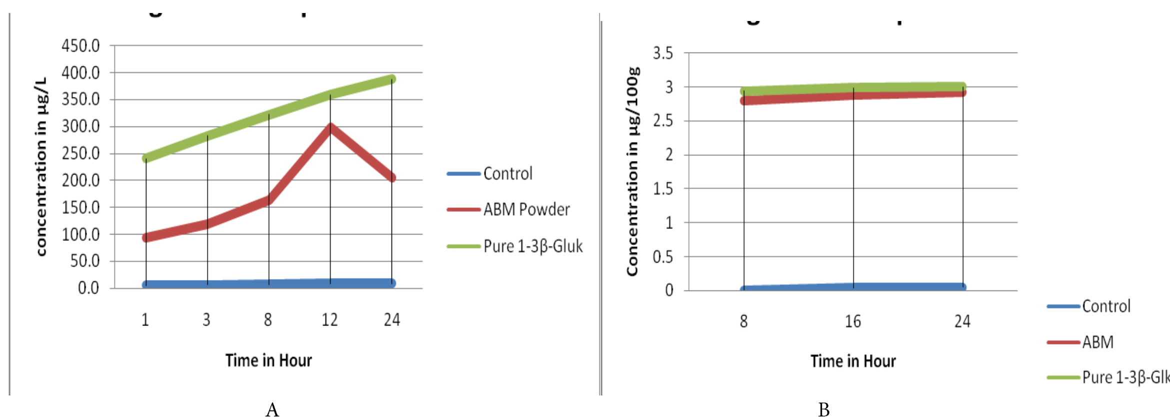


Figure 1. Graph the average levels of 1,3-â-Glucan in Serum (ug / L) with pure compound treatment, Agaricus blazei Murill powder, and control (A); Graph the average levels of 1,3-â-Glucan in Feces (ug / 100g) with pure compound treatment, Agaricus blazei Murill powder, and control (B)

Table 1. ANOVA

Source	Type III Sum of Square	df	Mean Square	F	Sig
Corected Model	856569.618	14	61183.544	354.820	.000
Intercept	1266062.941	1	1266062.941	7.342E3	.000
Treathment Time	75379.493	4	18844.873	109.286	.000
Treathment	730312.216	2	365156.108	2.118E3	.000
Treathment Time*treathment	50877.910	8	6359.739	36.882	.000
Error	5173.066	30	172.436		
Total	2127805.628	45			
Corrected Total	861742.684	44			

a.R Squared= .994(Adjusted R Squared = .991)

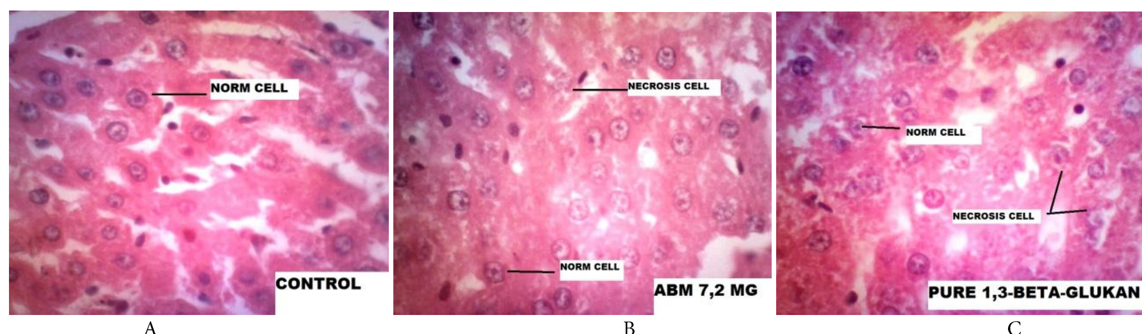


Figure 2. Staining with HE with 400x magnification, cells undergoing necrosis in the Livers: (A) Control; (B) 7.2 mg ABM; (C) 1.3-β-D-glucan pure compound

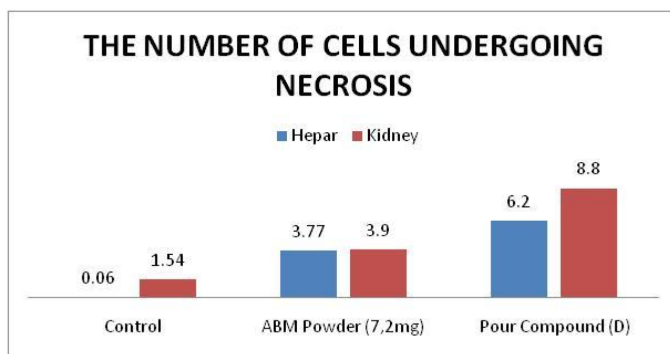
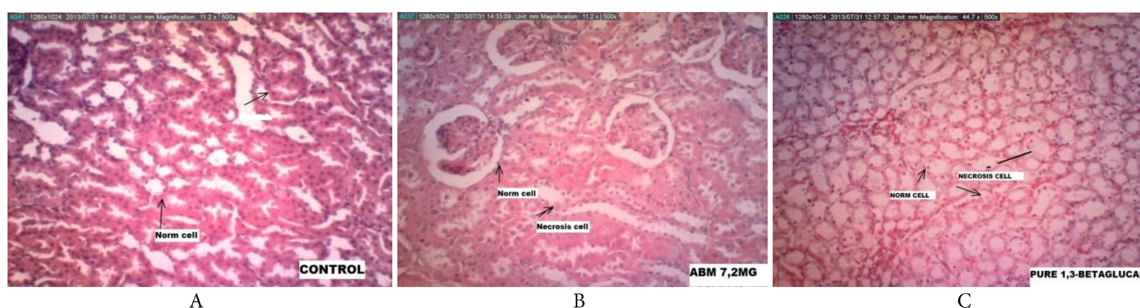


Figure 3. The number of cells undergoing necrosis in the livers and kidneys with control, ABM powder, and 1.3-β-D-glucan pure compound treatment

Table 2: ANOVA

	Sum of Squre	df	Mean Square	F	Sig
Between Group	51.857	4	12.964	48.654	0.000
Within Group	2.665	10	0.266		
Total	54.22	14			

Sig (0.000) < 0.05



A B C

Figure 4. Transverse slices of Kidney with HE staining 400x magnification. Control treatment (A), ABM powder 7.2mg treatment, and Pure 1,3-Beta-Glucan

Table 3. ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	93 177	4	23 294	26 284	.000
Within Groups	8862	10	.886		
Total	102 039	14			

Sig (0,000) < 0,05

Next, the levels of 1.3-β-D-glucan in ABM powder differ significantly from that of the control group and the levels approach the level of 1.3-β-D-glucan pure compound. This means that the powder has similar activities to the 1.3- β-D-glucan pure compound. Based on this, since the levels are not different much between the 1.3-β-D-glucan in ABM powder weighing 7.2 mg and that in pure compound weighing 1mg, the use of ABM mushroom powder is cheaper and easier to use. Also, since the delivery of 1.3-β-D-Glucan of pure compound is faster than that of the ABM powder which goes to the receptors faster, the therapeutic or toxic effect of the compound is also faster, depending on the dose given. Therefore, the use of 1.3-β-D-glucan pure compound should be provided with more caution and overview in regard to the dose given. This suggests that the use of ABM powder is relatively safer than that of the 1.3-β-D-glucan pure compound. The evidence of this can be inferred from the histopathological observations of liver and kidney after control, ABM powder, and 1.3-β-D-glucan pure compound. It was shown that there are more cells undergoing necrosis in the 1.3-β-D-glucan pure compound group than that in the control and ABM powder. Necrosis itself shows the cells which experience permanent damages as a result of a treatment.

Levels of 1.3-β-glucans in the urine samples after 8 hours, 16 hours, and 24 hours of treatment (control, ABM powder, and 1.3-β-D-glucan pure compound) show a negative result. This indicates that the compounds have experienced perfect metabolism. The active substance that

functions as a drug in the body will experience certain phases: absorption, distribution, metabolism, and elimination. The metabolism process of 1.3-β-D-Glucan experiences hydrolyses and changes into glucose (Tania *et al.,* 2008). This process occurs in the first phase of metabolism which occurs in the liver. The next phase after metabolism is elimination. The elimination phase is the process of excretion after metabolism. Here, the 1.3-β-D-Glucan experiences hydrolysis which leads to the process of excretion in the urine contains no 1.3-β-D-glucan compound.

Meanwhile, the levels of 1.3-β-D-Glucan in each feces samples decreases through time. Comparison between control, ABM powder, and 1.3-β-D-glucan pure compound in the feces showed significant differences between the treatments. The highest level of 1.3-β-D-Glucan in a certain time span is in its pure compound. This suggests that during the process of metabolism in the liver there is some 1.3-β-D-glucan which does not experience metabolism and some do not experience the process of absorption so it is not excreted through feces.

Based on the result can be concluded that the dynamics of the 1.3-β-D-glucan compound which was given in the form of ABM powder in the rats' blood samples showed an increasing level of 1.3-β-D-Glucan in 1 hour, 8 hours, 12, hours after treatment, whereas at 24 hours after treatment the level of 1.3-β-D-glucan decreased. When compared with the 1.3-β-D-Glucan pure compound group, after 1 hour, 8 hours, 12 hours, and 24 hours of treatment there has not been any decrease in the levels of 1.3-β-D-glucan. In the urine samples there were no 1.3-β-D-glucan compound found in the treatment of ABM powder in that of 1.3-β-D-glucan pure compound. While in the feces samples there were 1.3-β-D-Glucan found in all treatments. The effects on the kidney and liver histopathology showed that cells which underwent necrosis were found to be more in the treatment of 1.3-β-D-Glucan pure compound than that in the treatment of ABM powder, both in the kidneys and livers.

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