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Full Length Research Paper

The effect of *Agaricus blazei* Murill on haematological parameter, random blood sugar, total cholesterol, and uric acid of wistar rats (Sprague Dawley)

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***Agaricus blazei* Murill (ABM) has traditionally been used as a prevention and cure of several diseases, such as cancer, diabetes, hypertension, immunostimulant, due to its contents such as 1,3-β-D-Glucan, ergosterol, agaritine, tocoferolum, scorbic acid, terpenes. This study is carried out in order to uncover which doses of ABM powder treatment, classified as suitable doses for a healthy condition of experimental animals based on hematological parameters (haemoglobin, erytrocyt, and leucocyt), random blood sugar, cholesterol, and uric acid. This study was an experimental study using experimental animals (rats) treated with ABM powders (three doses) and pure compounds of 1,3-β-D-Glucan compared to the control group. Based on all parameter studied, it can be concluded that ABM consumption of 7.2 mg is the most suitable one of dose to be recomended for healthy condition.**

Key words: *Agaricus blazei* Murill, hematological parameter, random blood sugar, total cholesterol, uric acid.

INTRODUCTION

Agaricus blazei Murill (Agaricacea) known as ABM is a kind of mushroom that has been cultivated in Brazil, Japan, China, Korea, and Indonesia. ABM has traditionally been used as a prevention as well as a cure effort against cancer (Takaku et al., 2001; Yoshimura et al., 2005). Scientific research shows that the functions of this mushroom among others are as antidiabetic agent (Kim et al., 2005; Naso et al., 2010; Misgiati and Suprihartini, 2012), antiviral agent (Faccin et al., 2007; Firenzuoli et al., 2008; Wu et al., 2011), immunomodulator (Ooi and Liu, 2000; Lin et al., 2012), and antioxidant (Huang and Mau, 2006; Rhaoupas et al., 2010; Akiyama et al., 2011). The chemical components contained in ABM are polysaccharide compounds such as 1.3-β-D-Glucan, 1.6-β-D-Glucan, and glucan-protein (Naso et al., 2010), as well as minerals such as iron, potassium, phosphorus, magnesium, copper, selenium, manganese, and zinc (Novaes et al., 2007). According to Huang and Mau (2006), Al-Dbass et al. (2012) and Andreia et al. (2013), the mushroom also contains ascorbic acid and tocopherol (vitamin E), and agaritine (Rhoupas et al., 2010; Akiyama et al., 2011)

ABM is normally consumed by people either in a healthy or ill condition. The use of this mushroom for sick people is generally based on empirical information of the surrounding communities and as well as on scientific evidence. The scientific evidence is essentially based on the studies of the pharmacological activities of ABM. The chemical components contained in ABM promote a specific activity. The chemical components of ABM are then studied restricted to single components; 1.3-β-D-Glucan component has been found to act as an anticancer (Naso et al., 2010; Lee et al., 2008), 1.3-β-D-Glucan has a chemiostatic activity, cytolytic activity in human tumor cells (Novaes et al., 2007; Chan et al., 2009), as well as antimutagen and antitumor immune stimulation (Naso et al., 2010). Meanwhile, agaritine component also acts as an anticancer (Rhoupas et al., 2010; Akiyama et al., 2011).

In vitro research conducted on the anticancer ability

shows that god's mushroom has the ability to inhibit the growth of HeLa cell growth (Misgiati, 2012), to induce apoptosis by caspase activation followed by the release of cytochrome c from mitochondria in leukemia cell (Akiyama et al., 2011; Endo et al., 2010), to induce apoptosis through DNA fragmentation in LU99 human lung cells and KATU III gastric cancer cells (Hirohiko et al., 2008), to induce apoptosis via caspase-3 activity in androgen-independent PC3 cell/prostate cancer cells (Yu et al., 2009), to inhibit cell growth, to induce apoptosis in osteosarcoma HOS cell line and normal human osteoblast cell line (Wu et al., 2012), and to induce apoptosis in human myeloid leukemia cells (Kim et al., 2009). On the other hand, *in-vivo* studies related to the prostate cancer in rats uncover that tumor obstruction by ABM are occured by apoptosis and tumor growth inhibition by antiproliferation and antiangiogenesis (Yu et al., 2009).

As an antidiabetic agent (Misgiati and Suprihartini, 2012), ABM extract can lower the blood glucose levels in mice which are sterptozotocin induced. Beta glucan contained are immunostimulatory agent that can induce the formation of insulin (Misgiati and Suprihartini, 2012). According to Kim et al. (2005), β-Glucan treatment which is a chemical component of ABM can lower blood glucose in rats induced by streptozotocin. The mechanisms related are the increases of insulin secretion from islands of langerhans as well as the maintenance and proliferation of islands of langerhans cells of diabetic and normal mice.

As an antihipercholesterol agent (Kim et al., 2005), β–Glucan is effective in lowering blood cholesterol levels, but the mechanism related remains unclear (Nicolosi et al., 1999). According to Kim et al. (2005), ABM has an activity as antihypercholesterol agent in diabetic rats.

According to Ooi and Liu (2000) and Lin et al. (2012), *in-vivo* ABM can increase the immune response in rats suffering leukemia as well as can increase T-cell proliferation. ABM extract increases NK cell activity and phagocytosis of macrophages (Lin et al., 2012). As an antioxidant, components of natural antioxidant contained in ABM are ascorbic acid, α- tocopherol, δ-tocopherol, and total phenol (Andreia et al., 2013; Huang and Mau, 2006).

The use of ABM mushroom for healthy people is usually for complementary vegetables and precaution against diseases because this mushroom can maintain immunity against disease or function as an immunomodulator. In relation to the use of ABM mushroom for healthy people and as medicine (for its pharmacological activity), it is said that they are like two sides of a coin. On one side, it can be used for disease treatment and on the other side it can be used for supplement regarding its toxic effects. Therefore, a parameter to measure the effect of the mushroom for healthy people needs to be set up; the parameters referred here consist of the parameter for the level of hemoglobin, the amount of erythrocyte, leukocyte, and the level of random/direct blood glucose, total cholesterol, and uric acid.

MATERIALS AND METHODS

ABM

ABM was obtained from the cultivation of traditional medicine industry namely Sido Makmur Lawang, Malang, Indonesia. The Sido Makmur industry is located at an altitude of 667 m above sea level. ABM used collection in January 2013.

Pure compounds of 1,3-β-D-Glucan

The kind of 1.3-β-D- Glucan compound used is 1.3-β-D-Glucan Euglena gracilis (Sigma Aldrich, CAS: 9051-97-2).

Experimental animal

Experimental animals used were Sprague Dawley white rats which were male, 6-7 weeks old, weight of 200 g obtained from D'Wistar unit in Bandung, Indonesia. The number of rats used were 15, consisting of 3 rats as the control group, 3 rats treated by 3.6 mg ABM powder, 3 rats treated by 7.2 mg ABM powder, 3 rats treated by 18 mg ABM powder, and 3 rats treated by 1 mg of pure 1,3-β-D-Glucan. The rats were individually reared with a temperature of 25°C, undergoing 12 h of full light and 12 h of no light at all. The food given was in the form of pellets (PT Charon Phokpan, Indonesia), and the water was given *ad libitum*. Experimental animals were acclimatized for 7 days prior to the treatment.

Procedure

All parts of the mushroom were used, after dried and pulverized. Related to powders manufacturing processing, ABM were blended and then sieved. ABM powder used was of 3.8, 7.2 and 18 mg based on the conversion of the human body weight. In this connection, ABM needed is as much as 200 mg, and dose corresponding to 200 mg was 7.2 mg. ABM was dissolved in Aquadest of 1 ml and was administered orally. Oral administration was adapted to the use in the community. Pure 1,3-β-D-Glucan used of 1 mg was dissolved in 1 ml Aquadest, based on 1,3-β-D- Glucan contained in the ABM as much as 12% of the ABM content. Pure 1,3-β-D-Glucan was also administered orally. The experimental animals were divided as the control group and those treated with the ABM powder of

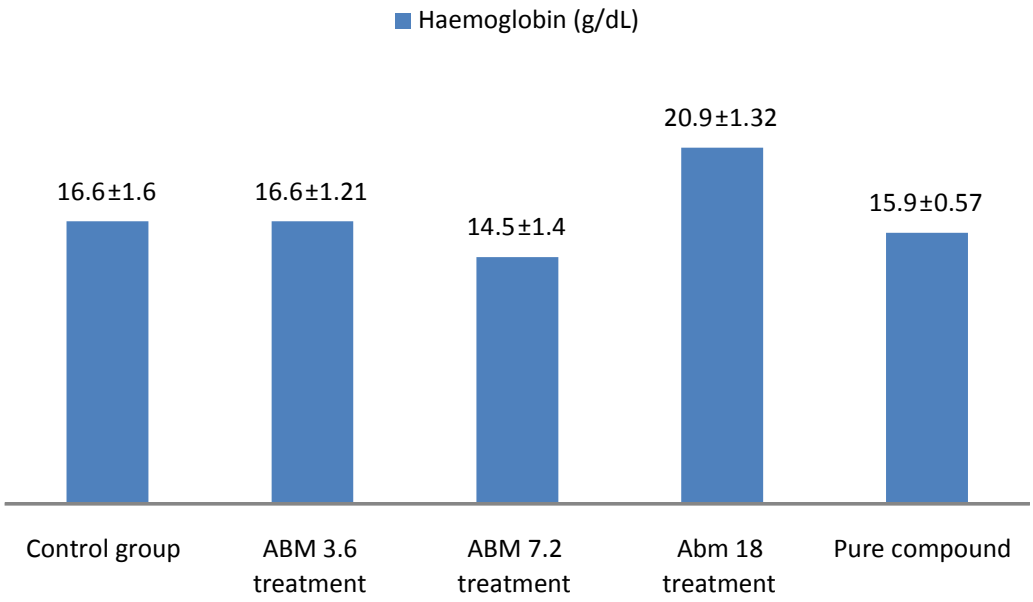

Figure 1. Hemoglobin levels detected in the control group, ABM powder, and in the treatment of the pure compound of 1,3-β-D-Glucan.

3.8, 7.2 and 18 mg, and 1.3-β-D-Glucan pure compound for 4 week. The treatment was conducted 7 times in a week, so the total treatments were 28 times. Furthermore, the blood of the rats was taken as much as 3-5 ml by cardiac puncture. Blood obtained was directly tested in central laboratory of Saiful Anwar Hospital, in Malang, Indonesia, related to parameter test of hematology and clinical chemistry. Animals were used in accordance with the ethical clearance sacrificed No. KEP-153-UB of Animal Care and Use Committee of the Brawijaya University, Indonesia.

RESULTS AND DISCUSSION

Hemoglobin

The levels of haemoglobin obtained are shown in Figure 1. Hemoglobin level of control group is 16.6 g/dL, but those of 3.6 mg ABM, 7.2 mg ABM, 18 mg ABM, and of the pure compound are 16.6, 14.5, 20.9 and 15.9 g/dL. Based on ANOVA test supported by SPSS 22, there is no significant difference among those haemoglobin levels obtained (p value = 0.067). Haemoglobin is a protein that is rich in iron. It has affinity to oxygen. With oxygen, haemoglobin forms oxyhaemoglobin in red blood cells. In other side actually, haemoglobin levels also affect the levels of erythrocytes, because AMB contains iron, folic acid, vitamin B12, copper, cobalt, and tryptophan which are able to stimulate erythrocyte (Schiau, 2013); it also contains ascorbic acid and tocopherol (Rhoupas et al., 2010; Akiyama et al., 2011) which can increase the levels of red blood cells in the blood (Sundaryono, 2011).

Erytrocyt

Based on the erytrocyt levels presented in Figure 2, the erytrocyt levels of control group, 3.6 mg ABM powder treatment, 7.2 mg ABM powder treatment, 18 mg ABM powder treatment, and of the treatment of the pure compund, are 6.91 x 10^6/mL, 6.99 x 10^6/ µL, 6.9 x 10^6/ µL, 7.16 x 10^6/ µL, and 7.12 x 10^6/ µL. Based on ANOVA test supported by SPSS 22, there is no significant difference (p value = 0.163): among all the groups. ABM contains vitamin B12, folic acid, iron, copper, cobalt, and tryptophan that can stimulate the production of red blood cells (Schiau, 2013); furthermore there are also antioxidants contained in ABM like Vitamin C and Vitamin E (Rhoupas et al., 2010; Akiyama et al., 2011). These antioxidants contribute in boosting the erythrocyte levels in the blood (Sundaryono, 2011). On the other hand, those ABM contents is secured having no effect on the increase of the erytrocyt production in healthy condition.

Leucocyt

Based on the leucocyt level presented in Figure 3, the leucocyt levels of control group, 3.6 mg ABM powder treatment, 7.2 mg ABM powder treatment, 18 mg ABM powder treatment, and of the treatment of the pure compound, are 3.71 x 10^3/µL, 3.67 x 10^3/µL, 3.73 x 10^3/µL, 3.68 x 10^3/µL, and 3.76 x 10^3/µL. Based on ANOVA test supported by SPSS 22, there is no significant diference (p value = 0.535) among the groups, including the control group and those of the treatment groups. In this connection, the provision of ABM powder

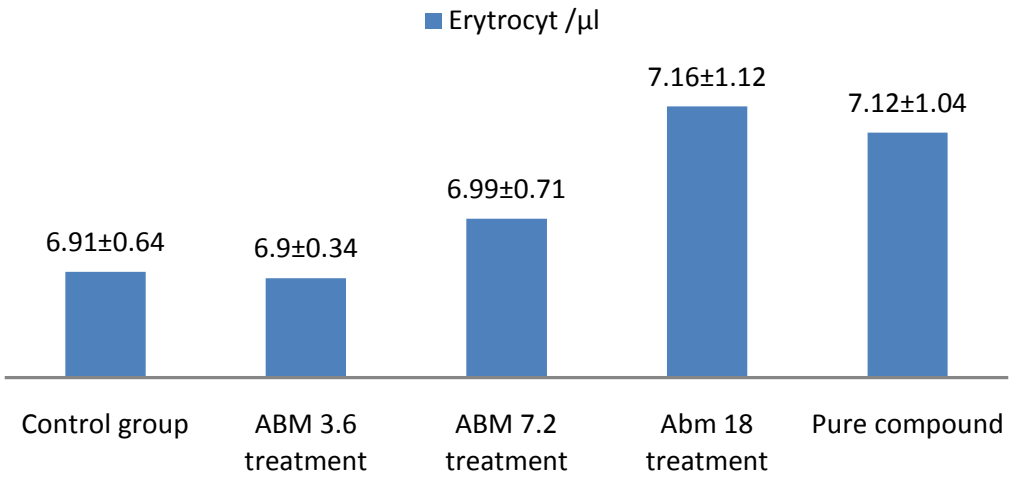

Figure 2. Erytrocyt levels detected in the control group, ABM powder treatment, and the pure compound of 1,3-β-D-Glucan.

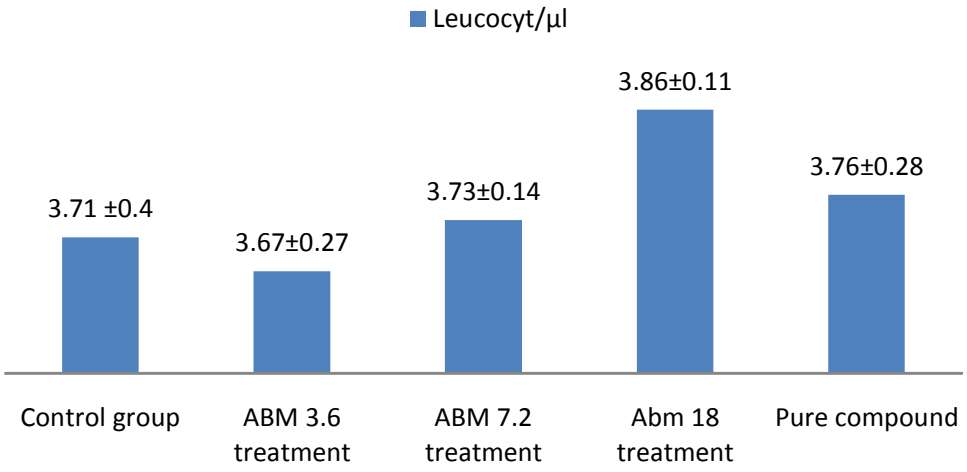

Figure 3. Leucocyt levels detected in the control group, ABM powder treatment, and in the treatment of the pure compound of 1,3-β-D-Glucan.

restricted to the three doses have no significant effect on the leucocyt level of the healthy mice. The leukocyte level in the body indicates the presence or the absence of any disease. The ABM powder has an activity as immunomodulator by restoring the immune system imbalance (Ooi and Liu, 2000; Lin et al., 2012), while the components that have an activity as an immunomodulator is 1,3-β-D-glucan, 1,6-β-D-glucan, and glucan protein (Naso et al., 2010). The ABM powder would reduce the number of leukocytes in a body having a bad immune system condition, but in a healthy condition the ABM powder had no effect on the level of leukocytes. Therefore related to leucocyt level, this research result is in line with Ooi and Liu (2000) and Lin et al. (2012).

Random blood glucose

Based on the level of random blood glucose presented in Figure 4, the random blood glucose of control group, 3.6 mg ABM powder treatment, 7.2 mg ABM powder treatment, 18 mg ABM powder treatment, and of the treatment of the pure compound, are 80, 75.5, 92.5, 71.5 and 70.5 mg/dL. Based on ANOVA test supported by SPSS 22, there is no significant diference (p value = 0.128) among the groups, including the control group and those of the treatment groups. It means that ABM treatment of the three doses does not affect yet the random blood glucose of healthy rats. On the other hand, according to Misgiati and Suprihartini (2012), ABM could lower blood glucose levels in mice induced with streptozotocin

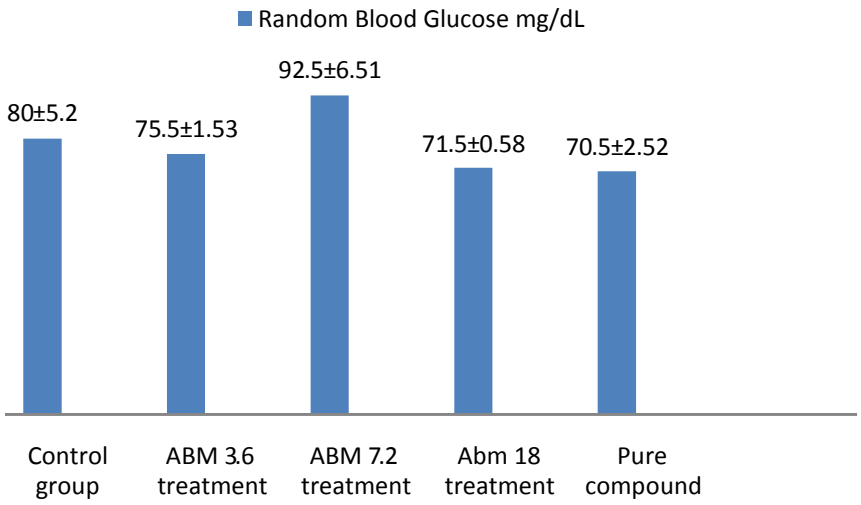

Figure 4. Random blood glukose levels in the control group, ABM powder treatment, and in the treatment ofthe pure compound.

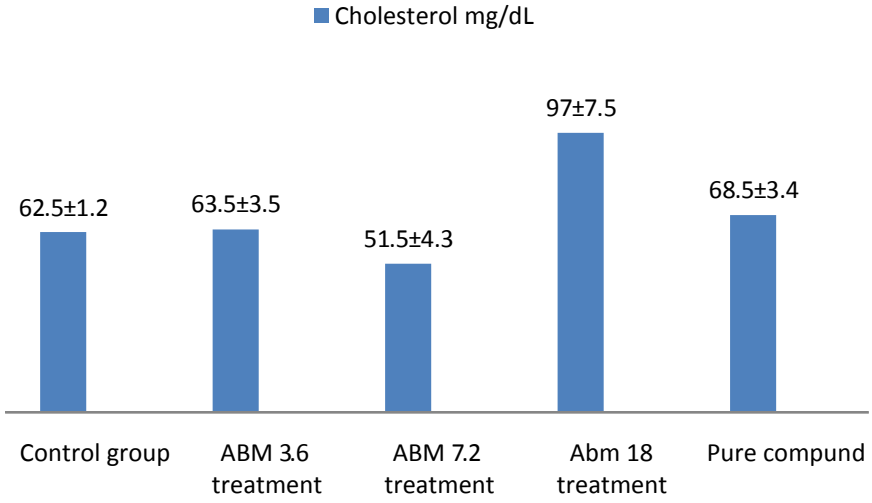

Figure 5. Total Cholesterol levels detected in the control group, ABM powder treatment, and in the treatment of the pure compound of 1,3-β-D-Glucan.

(diabetic mice) and had a significant difference with the positive control group (glibenclamide). In contrast, according to Kim et al. (2005), 1,3-β-D-Glucan could lower blood glucose levels in rats by increasing the insulin secretion from the island of langerhans, as well as by increasing the survival and cell proliferation in the island of langerhans both in normal rats and in streptozotocin-induced-rats powder group. Despite ABM would still have an activity of lowering blood glucose levels in diabetic condition, however, based on this research result it can be stated that the ABM mushroom consumption by people in a healthy condition had no effect on random blood glucose level. Furthermore, based on the random blood glucose levels result, the recommended intake of ABM was 7.2 mg, because the glucose levels in control was 80 mg. The glucose levels of another treatments are leading to hypoglycemic condition, although there is no significant difference yet.

Total cholesterol

Based on the total cholesterol levels presented in Figure 5, the total cholesterol levels of control group, 3.6 mg ABM powder treatment, 7.2 mg ABM powder treatment, 18 mg ABM powder treatment, and of the treatment of the pure compound, are 62.5, 63.5, 51.5, 97 and 68 mg/dL. Based on ANOVA test supported by SPSS 22, there is significant diference (p value = 0.004) among the groups, including the control group and those of the

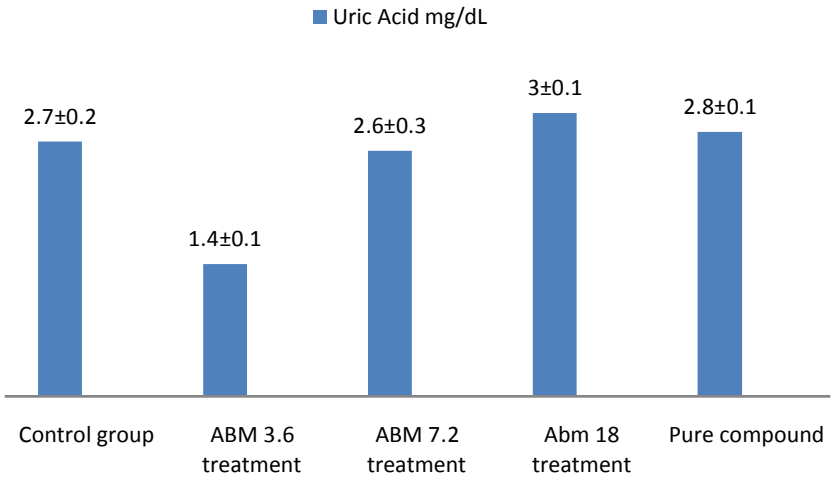

Figure 6. Uric acid levels detected in the control group, ABM powder treatment, and in the treatment of the pure compound of 1,3-β-D-Glucan.

treatment groups. It means that any treatment group (one or more) have significant effect compared to the control group effect. The 18 mg ABM has a significant difference of total cholesterol and a higher on compared to all other treatments or group (control, 3.6 mg ABM, 7.2 mg ABM, and 1,3-β-D-Glucan pure compound). The total cholesterol of 7.2 mg ABM treatment is lower and has a significant difference compared to the total cholesterol of 1,3-β-D-glucan pure compound; but it has no significant diferrence compared to those of control group and of 12 mg ABM. It can be said that the ABM powder treatments (except that of 7.2 mg ABM) give higher levels of total cholesterol compare to those of the 1,3-β-D-Glucan pure compound and of control group. This fact could be caused by more food consumption observed in rats treated with ABM powder compared to the others. According Takaku et al. (2001) and Novaes et al. (2007), in ABM there are Vitamin B1, Vitamin B2, and Niacin as the components of vitamin B complex. Vitamin B complex increases appetite. On the contrary, according to Kim et al. (2005), ABM has an antihypercholesterol activity for diabetic rats. ABM has an activity of lowering cholesterol levels in diabetic condition, but in a healthy condition it will improve cholesterol levels. It can be stated that 3.6 mg and 7.2 mg ABM are the suitable doses to be purposed for a healthy condition.

Uric acid

Based on the uric acid levels presented in Figure 6, the uric acid levels of control group, 3.6 mg ABM powder treatment, 7.2 mg ABM powder treatment, 18 mg ABM powder treatment, and of the treatment of the pure compound, are 2.7, 1.4, 2.6, 3 and 2.8 mg/dL. Based on ANOVA test supported by SPSS 22, there is significant diference (p value = 0.002) among the groups, including the control group and those of the treatment groups. It means that any treatment group (one or more) have significant effect compared to the control group effect. Uric acid is the end-product of purine metabolism in the body. Under normal circumstances, there is a balance between the formation and degradation of purine nucleotides and the ability of kidneys to excrete uric acid. If there is excess formation (overproduction) or decreased excretion (underexcretion) or both, there will be an increase in the concentration of uric acid in the blood called hyperuricemia. The treatment of ABM powder especially related to 7.2 mg and 18 mg show that their uric acid levels are not significantly different with the uric acid level of the control group. The uric acid level of 3.6 mg ABM treatment is different significantly and lower compared to uric acid level of the control group. Therefore related to uric acid level, ABM consumptions of 7.2 mg and 18 mg are suitable be proposed for healthy condition. ABM consumption in healthy condition should be considered with regard to the increase of uric acid levels. This is due to fact that in the ABM there is Molybdenum that can accelerate the absorption of glutamine and threonine, as well as can regulate metabolism which can result in uric acid production (Schiau, 2013).

Conclusion

ABM powder treatments (related to the three doses) have no effect on the levels of hemoglobin, erytrocyt, leucocyt, and random blood glucose of healthy rats. On the other hand related to total cholesterol level, the ABM powder treatments (except that of 7.2 mg ABM) give higher level total cholesterol compared to those of the of 1,3-β-D-Glucan pure compound and of control group. In this connection, 3.6 mg and 7.2 mg ABM are the proposed

doses for a healthy condition. Furthermore, ABM powder treatments (especially related to 7 and 18 mg) affect the same uric acid levels compared to those of control group and of 1,3-β-D-Glucan pure compound. Therefore it can be concluded that related to uric acid level ABM consumptions of 7.2 mg and of 18 mg are suitable be proposed for healthy condition. Finally based on all parameter studied, it can be concluded that, ABM consumption of 7.2 mg is the most suitable one of dose to be recomended for healthy condition.

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