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THE EFFECT OF PASAK BUMI ROOTS TOWARDS BLOOD GLUCOSE LEVEL IN GLUCOSE-LOADED MICE

EFEK PEMBERIAN AKAR PASAK BUMI TERHADAP KADAR GLUKOSA DARAH PADA MENCIT TERBEBANI GLUKOSA

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ABSTRACT

The aim of this research is to evaluate the effect of pasak bumi roots (Eurycoma longifolia Jack) towards the blood glucose level in glucose-loaded mice. The blood glucose-lowering effects were tested using Oral Glucose Tolerance Test (OGTT) method. The mice were given with infusion of pasak bumi roots at the doses of 0.83; 1.67; 3.33 g/kgBW and methanol extract of pasak bumi roots at the doses of 102; 210; 420 mg/kgBW. All treatments were conducted orally, 30 minutes before the administration of glucose (2 g/kgBW). The blood glucose levels were measured at 0 minute before the administration of glucose and at 15, 30, 60, 90, and 120 minutes after the administration of glucose. Blood samples were obtained through the mice tail's vena lateralis using glucometer. The blood glucose levels result which were obtained at the 0 until 120 minutes were calculated to obtain AUC. AUV values of each treatment group were analyzed statistically. Based on the results of the research, it can be concluded that the methanol extract of pasak bumi roots has blood glucose-lowering effect at the doses of 210 and 420 mg/kgBW, but infusion of pasak bumi roots does not have effects on lowering the blood glucose level in the glucose-loaded mice.

Keywords: *blood glucose, Eurycoma longifolia Jack, oral glucose tolerance test*

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh pemberian akar pasak bumi (Eurycoma longifolia Jack) terhadap kadar glukosa darah pada mencit yang terbebani glukosa. Efek penurunan kadar glukosa darah menggunakan metode uji toleransi glukosa oral (UTGO). Sejumlah mencit diberikan infusa akar pasak bumi dengan dosis 0,83; 1,67; 3,33 g/kgBB berturut-turut dan ekstrak metanol akar pasak bumi dengan dosis 102; 210; 420 mg/kgBB. Semuanya diberikan secara per oral, 30 menit sebelum pemberian glukosa (2g/kgBB). Kadar glukosa darah ditetapkan pada menit ke-0 sebelum pemberian glukosa dan pada menit ke-15, 30, 60, 90, dan 120 setelah pemberian glukosa. Pengambilan darah dilakukan melalui vena lateralis ekor pada mencit menggunakan glukometer. Hasil kadar glukosa darah yang didapat pada menit ke-0 sampai 120 dihitung AUC. Data AUC tiap kelompok perlakuan dianalisis secara statistik. Dari hasil penelitian disimpulkan bahwa ekstrak metanol akar pasak bumi memiliki efek penurunan kadar glukosa darah pada dosis 210 dan 420 mg/kgBB sedangkan infusa akar pasak bumi tidak memiliki efek untuk menurunkan kadar glukosa darah pada mencit yang terbebani glukosa darah.

Kata kunci: *glukosa darah, Eurycoma longifolia Jack, uji toleransi glukosa oral*

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease or metabolism disorder with multi etiology which is characterized by high blood glucose levels along with carbohydrate, lipid and protein metabolism disorder as a result of insufficient function of insulin. Insufficient level of insulin can be caused by a disorder or deficiency of insulin production by the Langerhans beta cells of the pancreas gland, or caused by the lack of responsiveness of body cells to insulin (Ministry of Health of Republic of Indonesia, 2005). According to the latest estimate of the IDF (International Diabetes Federation) there are 382 million people living with diabetes in the world in 2013 and by 2035 it is estimated that the number will increase to 592 million people. From year to year, the prevalence of *diabetes mellitus* continues to increase (Kemenkes RI, 2014).

In this modern era, the use of plants as an alternative treatment is still practiced by the community because it is considered that traditional medicine has less side effects than synthetic medicine and does not require a lot of cost (Kuntorini, 2005). *Pasak bumi* roots (*Eurycoma longifolia Jack*) is a plant which is widely used in traditional medicine; one of them is as antidiabetes (Rehman et al., 2016). Khanam et al. (2014) reported that *pasak bumi* roots contain phenolics, flavonoids and terpenoids. The compounds responsible for lowering blood glucose levels are flavonoids, tannins, triterpenoids and steroids (Kaimal et al., 2010). Flavonoids are polyphenol compounds which are found in many plants. Flavonoids can work by inhibiting the sodium dependent glucose transporter (SGLT 1), thereby limiting the entry of free glucose to the system. Glucogenic enzymes are also inhibited by flavonoids to decrease the rate of gluconeogenesis pathways, which involve the biosynthesis of glucose from non-carbohydrate sources. In addition, flavonoids can increase the glucose uptake by the cells using GLUT4 and thereby can reduce the free glucose in the system (Afroz et al., 2016).

Husen (2004) reported that *pasak bumi* in the freeze-dried form can give effect to lower the blood glucose level. Therefore, in this research, the infusion form is selected, because it is a practical form that can be used by the community. In addition, the form of methanol extract is also selected because it is known that methanol can attract flavonoid compounds that can lower blood glucose levels (Khanam et al., 2014). In this research, it is expected that the compounds contained in *pasak bumi* roots that can reduce glucose levels will be optimally filtered through infusion and extraction with methanol. The existence of this research is expected to figure out the effect of infusion and methanol extract of *pasak bumi* roots in lowering the blood glucose level in the glucose-loaded mice.

METHODS

Materials and Instrumentation

Materials used in this research were Swiss male mice weighing 20-30 grams, aged 2-3 months, the *pasak bumi* root obtained from PT Merapi Farma Herbal Yogyakarta and has been determined in Faculty of Biology. UGM, Yogyakarta, glucose (Merck®), 95% methanol (Merck®), distilled water, CMC Na (Merck®), blood glucose test strip (GlucoDr® auto). The equipments used were the analytical scales (Mettler Toledo®), oral injection syringe of 1 cc (Terumo®), GlucoDr® auto glucometer, lancet, glassware (pyrex®), mesh sizes 40 and 50, oven (Memmert), moisture balance equipment, pollinating machine (Retsch), heater, enamel pan, flannel cloth, thermometer, waterbath, rotary evaporator (Buchi®).

Production of *Pasak Bumi* Roots Powder Infusion

Ten grams of *pasak bumi* roots powder was weighed, then 100 mL of distilled water was added and they were mixed inside an infusion vessel. The mixture was heated over the water bath for 15 minutes with 90°C of temperature. The 15-minute time was calculated when the temperature of the mixture reached 90°C. The mixture was squeezed using the flannel cloth, and then hot water was added sufficiently through the dregs

to obtain 100 mL (Directorate of Original Medicines of Indonesia, 2010).

Production of *Pasak Bumi* Roots Methanol Extract

A total of 10 g of dried powder of *pasak bumi* roots that were filtered, was extracted with 100 mL of 95% methanol solvent at the room temperature for 48 hours by maceration (Hendra et al., 2017). The extract obtained (yield of 1.89% w / w) was then dispersed in 1% CMC-Na.

Classification and Treatment of the Test Animals

A total of 40 mice were divided into 8 groups randomly. Prior to treatment, the test animals were not given any food for 16-18 hours but were still given water to drink. Group I was given distilled water at a dose of 25 g/kgBW. Group II was given glucose at a dose of 2 g/kgBW (Ikarashi et al., 2011; Mudgal et al., 2016). Groups III, IV, and V were given *pasak bumi* roots infusion (IAPB) with three dose ratings of 0.83; 1.67 and 3.33 g/kgBW in sequence. Groups VI, VII and VIII were given methanol extract of *pasak bumi* roots (EMAPB) at a dose of 105; 210 and 420 mg/kgBW respectively (Hendra et al., 2017). All of them were aministered orally. Time of infusion and methanol extract of *pasak bumi* roots was 30 minutes before the glucose was administered (Hasanah et al., 2016 and Chaimum-aom et al., 2017). This research has been approved by the Ethical Clearence of Universitas Gadjah Mada (KE/FK/0794/EC/2017).

Determining the Blood Glucose Level

The blood glucose level in glucose-loaded mice using oral glucose tolerance test (OGTT) was measured at minute 0 before glucose was administered and at minute 15, 30, 60, 90 and 120 after glucose was administered. Blood was taken through the *vena lateralis* of the mice tail and blood glucose levels were measured using glucometer. After blood glucose levels were obtained, a blood glucose level value vs the minute 0 to 120 curve was created using the trapezoid method (AUC_{t0-tn}) and the formula (Mustaffa et al., 2014) used was as follows:

$$AUC_{t0-tn} = \frac{t_1 - t_0}{2} x(C_0 + C_1) + \frac{t_2 - t_1}{2} x(C_1 + C_2) + \frac{t_n - t_{n-1}}{2} x(C_{n-1} + C_n)$$

Note:
t = time (minute)
C = glucose level in blood (mg/dL)
AUC_{t0-tn} = area under the curve from 0 minute until n minute

Analysis of the Results

The AUC_{0-120} blood glucose data were analyzed statistically. It was started with the Shapiro-Wilk test to find out whether the data were distributed normally or not as a requirement of parametric analysis. If the data were not distributed normally, then it would be analyzed by using Kruskal Wallis test to figure out the difference between each group. After that, it was continued with the Mann Whitney test to find out the significance of the differences of each group. However, if the data are normally distributed, it would be continued by the analysis of one way variance pattern (One Way ANOVA) with 95% of validity level. Furthermore, Tukey HSD test would be conducted if the data were homogeneous and Tamhane test would be employed if the data were not homogeneous.

RESULTS AND DISCUSSION

Based on the results of the research presented in Figure 1, the curve of the correlation between time and average blood glucose level of each treatment was obtained. It can be seen that the negative control group showed that the average of blood glucose level from minutes 0 to 120 is relatively unchanged. This indicates that the glucose levels of test animals in the negative control group showed no increase or decrease in blood glucose levels.

The glucose control group that was given 2 g/kgBW of glucose showed the highest blood glucose levels average at the 15th minute compared to the negative control group. This is consistent with the results of the research from Ikarashi et al. (2011), Mudgal et al. (2016) and Wongnawa et al. (2014) that

blood glucose levels will increase after 2g/kgBW of glucose is given. The results also showed that blood glucose levels reached its peak at the 15th minute, then began to decline in the 30th minute after the oral glucose was administered. This corresponds to the theory that the peak of the initial phase of glucose is the first 15-30 minutes after consuming glucose (Ernsberger & Koletsky, 2012). From Figure 1, it can be seen that 2 hours after the glucose was administered, the blood glucose levels started to return to normal. This is in accordance with the theory of Chee and Fernando (2007) that after being charged with glucose solution, blood glucose levels quickly return to normal conditions generally within 2 hours after the glucose administration. This indicates that the bodies of the tested animals are in good health because the tested animals can still tolerate the UTGO glucose loading at normal levels.

The results of post hoc test in Table I show that the IAPB treatment group at the doses of 0.83; 1.67 and 3.33 g/kgBW had no significant difference ($p > 0.05$) with the glucose control group. This suggests that the three dose ratings do not have the effect to lower the blood glucose levels. The results of the research showing that IAPB lacks the ability to lower blood glucose levels are suspected to be associated with the amount of flavonoid compound which was filtered using water solvents. The total number of flavonoids consumed using methanol solvent, ethanol or acetone is higher if compared to using only water on the leaves of Amomum chinense (Butsat & Siriamornpun, 2016) and Limnophila (Do et al., 2014). Therefore, it is necessary to identify the active compounds that are responsible for the activity of decreasing the blood glucose levels in mice.

Table I. AUC_{0-120} of Every Treatment Group

Treatment Group	Average of AUC_{0-120} ± SE
Glucose control	22618.50 ± 906.69[a]
Negative control	11317.50 ± 565.73[b]
IAPB dose at 0.83g/kgBW + glucose	20970.00 ± 740.75[a]
IAPB dose at 1.67g/ kgBW + glucose	19684.50 ± 1109.97[a]
IAPB dose at 3.33g/ kgBW + glucose	23215.50 ± 1265.73[a]
EMAPB dose at 105 mg/ kgBW + glucose	19221.00 ± 278.48[a]
EMAPB dose at 210 mg/ kgBW + glucose	17202.00 ± 988.52[a,b]
EMAPB dose at 420 mg/ kgBW + glucose	15274.50 ± 138.06[a,b]

Notes: SE: *Standard error*; a: $p<0.05$ shows significant difference towards the distilled water control group; b: $p<0,05$ shows significant difference towards the glucose control group; IAPB: *pasak bumi* roots infusion; EMAPB: *pasak bumi* roots methanol extract.

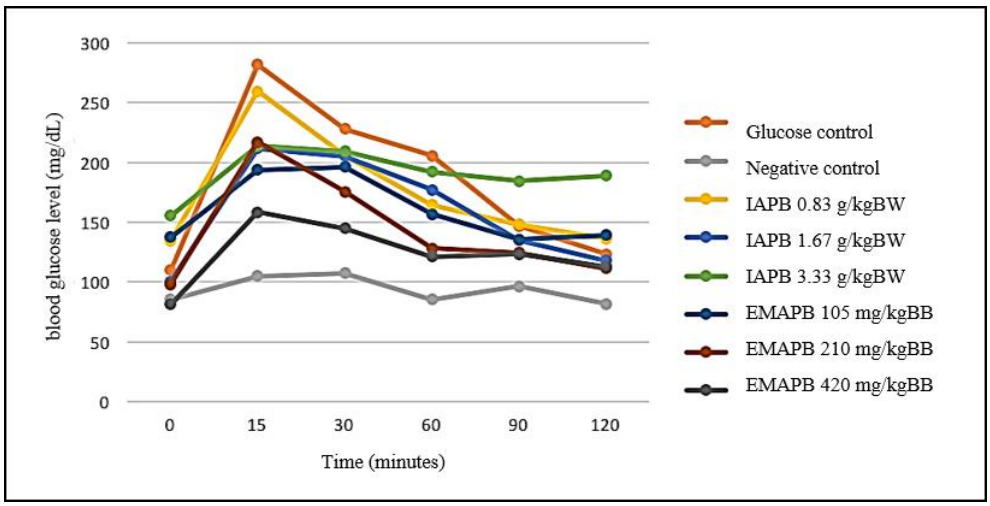


Figure 1. Correlation curve between time and the blood glucose level average in the *pasak bumi* roots infusion treatment (IAPB) and *pasak bumi* roots methanol extract (EMAPB)

The EMAPB treatment group at the dose of 105 mg/kgBW had an insignificant difference ($p > 0.05$) with the glucose control group, whereas the EMAPB treatment group at the doses of 210 and 420 mg/kgBW had significant differences ($p < 0.05$) against glucose control and negative control groups. This suggests that EMAPB administration at the dose of 105 mg/kgBW has no effect on lowering blood glucose levels. Administering EMAPB at the doses of 210 and 420 mg/kgBW has the effect to lowering the blood glucose in mice which are loaded with glucose but the decrease is not up to the normal levels. The ability to lower blood glucose from EMAPB at the doses of 210 and 420 mg/kgBW is due to the content of flavonoid compounds, which based on the research by Khanam et al. (2014), *pasak bumi* roots contain phenolic acid, flavonoids, and terpenoids. According to Lavle et al. (2016) flavonoids are polyphenol compounds which are present in plants, have antidiabetic effects by increasing insulin secretion, regulating glucose metabolism in hepatocytes, and increasing glucose uptake in skeletal muscle and adipose tissue. Flavonoids work by inhibiting sodium dependent glucose transporter (SGLT 1), thereby limiting the entry of free glucose to the system. Glucogenic enzymes are also inhibited by flavonoids to decrease the rate of gluconeogenesis pathways, which involve the biosynthesis of glucose from non-carbohydrate sources. In addition, flavonoids can increase glucose uptake by cells using GLUT4 and thus contribute to reducing free glucose in the system (Afroz et al., 2016). Flavonoids as antioxidants that have the ability to capture free radicals, also have the potential to have an antidiabetic effect (Oguntibeju, 2014). Lahrita et al. (2015) reported that the *pasak bumi* roots have the ability to increase insulin sensitivity in adipose, which plays an important role in the treatment of diabetes. Further research using the alloxan or streptozotosin induction model needs to be conducted to confirm the decreased activity of blood glucose level from methanol extract of the *pasak bumi* roots.

CONCLUSION

Administering the methanol extract of *pasak bumi* roots at the doses of 210 and 420 mg/kgBW has the effect of lowering the blood glucose levels, while the infusion of *pasak bumi* roots does not have the ability to lower blood glucose levels in glucose-loaded mice.

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