

## 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 (Mettmert), 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 administered 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 Clearance 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 ( $AUC_{t_0-t_n}$ )

and the formula (Mustaffa et al., 2014) used was as follows:

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

Note:

t = time (minute)

C = glucose level in blood (mg/dL)

$AUC_{t_0-t_n}$  = 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 30<sup>th</sup> 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<sub>0-120</sub> of Every Treatment Group

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

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.

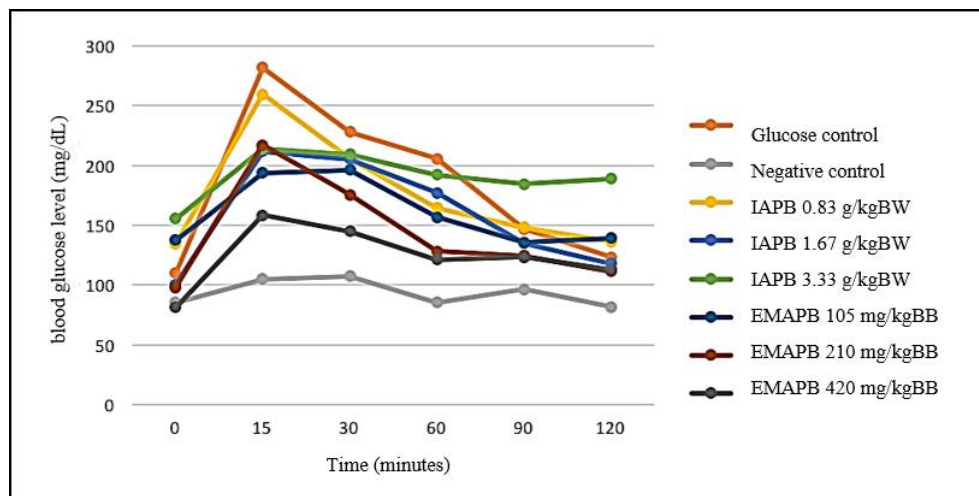


Figure 1. Correlation curve between time and the blood glucose level average in the *pasak bumi* roots infusion treatment (IAPB) and *pasak bumi* roots methanol extract (EMAPB)

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 streptozotocin 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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## QUALITATIVE ANALYSIS METHOD OF DETECTION OF WAX CONTENT IN GORENGAN USING SMARTPHONE

### METODE ANALISIS KUALITATIF DETEKSI KANDUNGAN LILIN PADA GORENGAN MENGGUNAKAN SMARTPHONE

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#### ABSTRACT

*Wax is one of the compounds that can be misused to be added to Gorengan, Indonesian fritter, to keep them crispy. Gorengan containing wax is difficult to identify visually, so a quick and easy method of detecting wax content is required. The purpose of this research is to develop and evaluate the analytical performance of detecting wax content in gorengan using smartphone. Gorengan sample was dissolved with hexane and then added reagent that will give discoloration followed by analysis using smartphone. Some analysis performance parameters were evaluated in terms of linearity and detection limit, qualitative analysis capability, precision, and selectivity test. The developed method was also applied in some gorengan samples. The result shows that the detection of wax content in gorengan can be conducted by using reagent consisting of NaOH, Schiff, and curcumin (1 : 2 : 2). Performance analysis shows that the linearity measurement at concentration between 10% and 25% has correlation coefficient (r) of 0.9537 with detection limit at concentration of 2% and precision (%RSD) less than 3%. The developed method can be applied for the detection of wax content in gorengan in the market.*

**Keywords:** analysis, chemometrics, gorengan, smartphone, wax

#### ABSTRAK

*Lilin merupakan salah satu bahan yang dapat disalahgunakan untuk ditambahkan pada gorengan agar tetap renyah. Gorengan yang mengandung lilin sulit untuk diidentifikasi secara visual, sehingga diperlukan metode deteksi kandungan lilin yang cepat dan mudah. Tujuan penelitian ini adalah untuk mengembangkan dan menguji kinerja metode analisis kandungan lilin dalam gorengan secara kolorimetri menggunakan smartphone. Penelitian ini merupakan penelitian eksperimental menggunakan gorengan sebagai objek penelitian. Gorengan dilarutkan dengan heksan dan kemudian ditambahkan reagen yang akan memberikan perubahan warna yang selanjutnya dapat dianalisa menggunakan aplikasi smartphone. Beberapa parameter kinerja analisis dievaluasi meliputi uji linearitas dan batas deteksi, uji kemampuan analisis secara kualitatif, uji presisi, serta uji selektifitas. Metode yang dikembangkan juga diaplikasikan untuk mendeteksi kandungan lilin pada beberapa sampel gorengan yang dijual di masyarakat. Hasil penelitian menunjukkan bahwa deteksi kandungan lilin dalam gorengan dapat dilakukan dengan menggunakan reagen yang terdiri dari NaOH, Schiff, dan kurkumin (1 : 2 : 2). Kinerja analisis metode yang dikembangkan menunjukkan bahwa linearitas pengukuran pada konsentrasi lilin 10% hingga 25% memiliki nilai koefisien korelasi (r) 0,9537 dengan nilai batas deteksi 2%*

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dengan nilai presisi (%RSD) kurang dari 3%. Metode yang dikembangkan dapat diaplikasikan untuk deteksi kandungan lilin pada sampel gorengan di pasaran.

**Kata kunci:** analisis, kemometrika, gorengan, smartphone, lilin

## INTRODUCTION

*Gorengan*, Indonesian fritter, is one of the favorite snacks among the Indonesia society. *Gorengan* can be bought easily because it is widely sold in the roadside (Chalid et al., 2008). The people like to eat *gorengan* because of its savory and crispy taste as well as cheap price. The increasing fondness of eating *gorengan* causes the high competition among *gorengan* vendors. This situation sometimes causes the vendors to sell only some portions of their *gorengan* product in one day. This problem certainly triggers the loss among the *gorengan* vendors and some of them try to find a way to keep their *gorengan* crispy and delicious eventhough it is stored for days. One of the ways is misusing of candle as food additives. Candle contains paraffin wax and others additives (Rezaei et al., 2002). The *gorengan* vendors deliberately add wax in a heated cooking oil when frying *gorengan*. The mixture of wax in heated cooking oil affects the crispy taste of *gorengan*. Wax is not a food additive and it is difficult to be broken down by enzymes in human body. Therefore, when wax is consumed, it can harm human's digestive function and cause health problems (Griffis et al., 2010; Derudi et al., 2014; Sjølling et al., 2018).

The content of wax in *gorengan* is difficult to identify visually. The analysis method of wax identification that is commonly used is gas chromatography associated with Mass Spectrometry, Near-infrared spectrometer (Palou et al., 2014), Differential Scanning Calorimetry (Chen et al., 2004; K k et al., 2007), High-performance Liquid Chromatography (Moreau et al., 2002), and Thin-layer Chromatography (Lu et al., 2008). These methods require expensive and complex equipment, detailed preparation and particular skill on its implementation. Therefore, it is necessary to develop a simpler, cheaper, easier

and more practical identification technique. A simple analysis technique commonly used in identifying a candle containing paraffin wax as the main compound is by using spot test analysis (Jungreis, 2004). Spot test analysis can be done by adding a particular reagent on the sample or by immobilizing the reagent in a membrane on the test strip and then the result is evaluated by identifying the discoloration (Ngom et al., 2010; Sutrisno et al., 2017).

The technology development has transformed smartphone not only to be a communication tool but also to be one of the identification tools of a compounds based on the color analysis of a figure or a sample. Smartphone is used in several studies as an analytical tool, such as detection of alcohol concentrations in saliva (Jung et al., 2015), detection of thiosulfate compounds using silver nanoparticles (Dong et al., 2017), formaldehyde measurement compounds in air (Yang et al., 2016), and others examinations. The potential use of smartphone for wax content detector in *gorengan* is important to be used by combining qualitative analysis of wax content based on the discoloration and the smartphone as the detector. This research aims to develop and examine methods of detection of wax content in *gorengan* by using smartphone. In addition, this research also aims to apply the developed method for qualitative analysis of wax content in *gorengan* sold in the market.

## METHODS

### Materials

The materials used in this research were candle, cooking oil, *gorengan*, aquadest, curcumin. The solvent and chemical compounds with pro-analysis quality were n-hexane, ethanol, NaOH, NaNO<sub>2</sub>, NaHCO<sub>3</sub>, Schiff's reagent (Merck Darmstadt, Germany).



### Instrumentation

The equipment used in this research were plat TLC Silica Gel GF<sub>254</sub> (Merck, Germany), TLC Chamber, capillary tube, vortex, micropipette 100µL-1000µL (Eppendorf, Germany), and 8 MP Smartphone camera (Oppo, China) with android operation of 5.1 ROI 32 x 32 pixel with *PhotoMetrix*<sup>®</sup> Figure analysis application version 1.2.1 (Ghelfer.net Inc., Brazil).

### Selection and Optimization of Reagents

The reagent in this research was curcumin 0.5%, NaOH 10%, Schiff, NaNO<sub>2</sub> 0.1% and NaHCO<sub>3</sub> 5% which were examined either single or multiple reagent combination. The test was conducted by dissolving wax in 2 mL of cooking oil that had been heated and then adding n-hexane solvent. As the negative control, the mixture of heated cooking oil and n-hexane solvent was used. On each oil solution, 500 µL of reagent was added and mixed to be homogeneous, then, the discoloration on each tube was observed. The selected reagent was a reagent giving clear discoloration when it was reacted. The selected reagent was then optimized in the form of volume of reagent and wax concentration in cooking oil in which the discoloration was observed as the next step. The test was conducted by dissolving wax in heated cooking oil and then adding n-hexane solvent and reagent. The most optimum reagent was the reagent giving proportional discoloration with the wax concentration added in cooking oil. It could be seen from the correlation coefficient (*r*) approaching ± 1 and providing clear and stable discoloration when it was analyzed in univariate analysis by using *Photometrix*<sup>®</sup> application on smartphone.

### Performance Test of Wax Analysis Method with Smartphone

Several performance tests of analysis method that were conducted are linearity test and detection limit, ability test in qualitative analysis, precision test, and selectivity test. Linearity test and detection limit were conducted by making seven concentrations of wax solution in cooking oil with the

concentration of 1% to 25 % with each three times replication. Two mL of wax solution from the cooking oil was poured into test tube containing 3 mL of n-hexane and 500 µL of reagent, and then, it was homogenized by using vortex. The colors formed were observed, captured by using *Photometrix*<sup>®</sup> application on smartphone, and analyzed through univariate analysis. The detection limit was calculated using linearity data based on the value of three times the residual deviation standard.

The test of method capability in qualitative analysis was conducted by examining the ability of analysis method in grouping cooking oil solution containing wax with different concentrations. In addition, the discoloration observation and capturing the figure by using smartphone was also conducted. Afterwards, it was analyzed through univariate analysis by using *Photometrix*<sup>®</sup> application. The precision test was conducted on self-made *gorengan* samples which is added with wax concentration of 0%, 5% and 10%. Each sample was replicated eight times. The sample was grinded and filtrated. Two mL of the sample was taken and it was mixed with 3 mL of n-hexane and 500 µL of reagent. Its discoloration was observed and the pixel value detected by *Photometrix*<sup>®</sup> application was evaluated. The calculation of %RSD was also conducted. Selectivity test was done by comparing cooking oil with 5% of wax concentration then fried dough was added. After it was prepared and reacted with reagent, the discoloration was observed and analyzed through univariate analysis using *Photometrix*<sup>®</sup> application. Afterwards, the calculation of measured wax content was done. The result was compared to the analysis of one-way ANOVA.

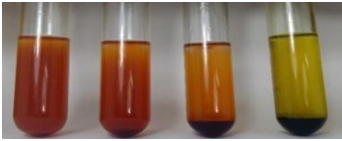
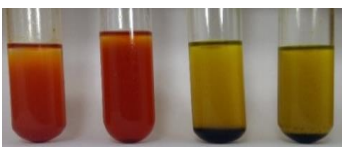
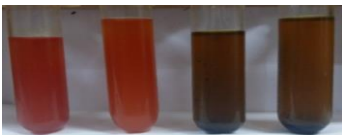
### Application Method on Gorengan Sample

The developed method was applied to identify wax content on several samples of *gorengan* sold by vendors which were randomly picked in the market. The types of *gorengan* analyzed in this research were fried tofu, fried *tempe*, fried banana, fried sweet

potato and *bakwan* (vegetables fritter snack). The samples of *gorengan* were prepared with n-hexane solvent and analyzed by adding 500 $\mu$ L of reagent and subsequently observed. The Figure was camptured by smartphone. A multivariate analysis using Photometrix<sup>®</sup> application was conducted to each sample of *gorengan* in order to identify the sample

classification into four groups; *gorengan* not containing wax, *gorengan* with low wax content, *gorengan* with medium wax content, *gorengan* with high wax content. The identification result of wax content in *gorengan* was then compared to TLC method using eluent of benzene : methanol (2.4 : 0.1).

**Table I.** The Result of Reagent Optimization for Wax Content Analysis in Cooking Oil

Ratio of Reagent Volume Variation				The color formed on the wax concentration in cooking oil			
Curcumin 0,5% ( $\mu$ L)	Schift ( $\mu$ L)	NaOH 10% ( $\mu$ L)	r	0%	1%	10%	20%
1	1	1	-0.784				
2	2	1	0.981				
1	2	2	0.637				
2	1	2	- 0.792				
3	1	1	0.863				
1	3	1	0.636				

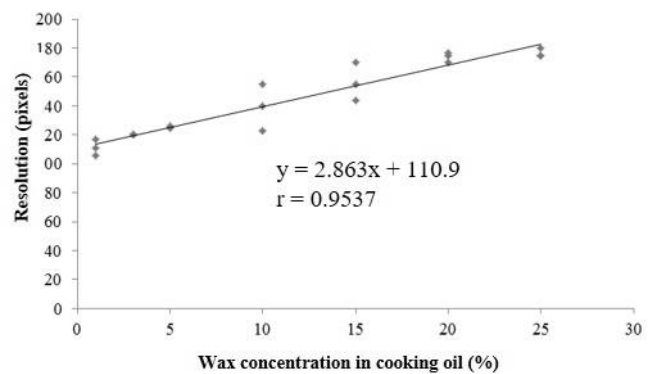
**RESULTS AND DISCUSSION**

**The Result of Selection and Optimization of Reagent**

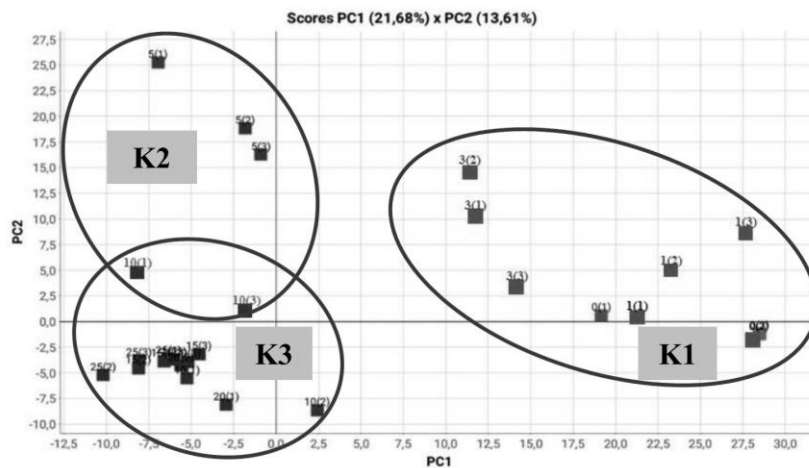
The examination result on several reagent which were examined either single or multiple reagent combination of curcumin 0.5%, NaOH 10%, Schiff, NaNO<sub>2</sub> 0.1% and NaHCO<sub>3</sub> 5% shows that optimal reagent can produce clear discoloration. Curcumin in the alkaline condition has a red colour (Bernabé-Pineda et al., 2004). After Schiff reagent was added, it strengthens the colour that is formed into a reddish blue. The presence of wax content will cause saponification reaction with NaOH. Therefore, it does not react to curcumin and will result curcumin reagent solution with yellow base. It is mixed with the reddish blue of Schiff reagent then resulting more concentrated green. Optimization of reagent is conducted in order to obtain reagent that can provide clearer and more stable discoloration. Optimization of reagent can be conducted by varying concentrations of curcumin, NaOH, and Schiff. The result of optimization of reagent is presented in Table I.

Based on the observation result of discoloration, there are selected ratios of reagent; curcumin 0.5%, Schiff 200 µL and

NaOH 10% (2:2:1). The ratio selection causes clearer and more stable discoloration after adding cooking oil containing wax and dissolved in an n-hexane solvent. The optimization of reagent is also based on the correlation coefficient (*r*) evaluated with univariate analysis. G channel on Photometrix® application is selected as measurement channel because it has *r* value approaching ± 1. This shows relationship between wax concentrations in cooking oil with color intensity that is produced (Helfer et al., 2017).



**Figure 1.** Linearity measurement of wax analysis in cooking oil using smartphone method



**Figure 2.** The analysis result of multivariate score plot of wax concentration using Photometrix® application

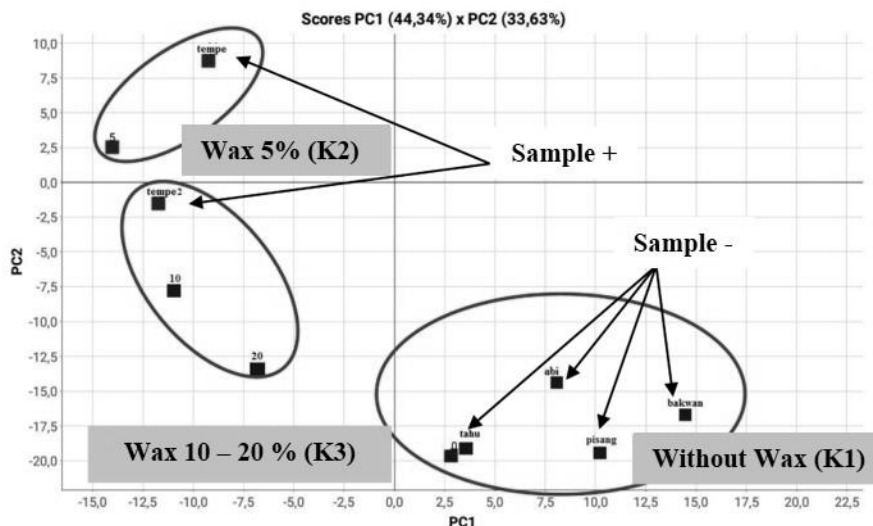


Figure 3. The result of score plot of wax content detection analysis of *gorengan* sample in the market using multivariate analysis and Photometrix® application on smartphone

**The Result of Wax Analysis Method by Using Smartphone.**

The result of linearity measurement of wax analysis in cooking oil was conducted to seven different wax solutions with 1% until 25% concentration detected by smartphone presented in Figure 1. Linear regression equation obtained is  $y = 2.863x + 110.9$  with correlation coefficient of  $r = 0.9537$ . The higher wax concentration in cooking oil will provide response analysis of color resolution recorded by smartphone presented in a proportional pixel unit. The calculation of detection limit by residual standard deviation method shows that wax concentration in cooking oil can still be detected by the developed method; 2.14%.

Qualitative analysis of the classification of wax sample in cooking oil with different concentration was conducted by multivariate analysis. This analysis helps to find out whether the wax which has similar concentration will be classified based on its classification. The classification test was conducted by using Principal Component Analysis (PCA). The variable category is the difference of wax concentration and evaluation on score plot presented in Figure 2. The result of score plot shows that the first and the second field has a total variability of

35.29%. PC1 and PC2 provide visualization of the separation among wax sample classifications in cooking oil with different concentration qualitatively. PC2 with 13.61% from the total variance is able to identify a sample into three groups; K1, K2 and K3. The first group, K1, consists of wax sample with concentration of 0%, 1% and 3%. The concentration in wax sample of K2 are 5% and 10%. While on K3, the concentration of wax sample are 10%, 20% and 25%. Due to the difference of quadrant position among wax with similar concentration, then PC selected horizontally able to separate among the groups. From the several PCs, PC1 with 21.68% of the total variance shows good result in separating among groups. K1 is on the positive side, K2 and K3 are on the negative side. However, K1 with 3% of wax concentration shows a tendency to approach the negative side. It is because the detection limit of this method is 2.14%. it can be concluded that PC1 and PC2 are proved to be able to classify wax standard based on the concentration. K1 is a group of wax standard with low concentration. K2 is a group of wax standard with medium concentration. While K3 is a group of wax standard with high concentration.

**Table II.** The Result of Precision Test of Analysis Method on Wax Concentration of 5% and 10%

Sample	Concentration of 0%	Concentration of 5%	Concentration of 10%
	(pixel)	(pixel)	(pixel)
1	97.50	125.00	135.00
2	102.50	128.00	138.10
3	102.00	128.00	133.50
4	95.00	125.00	141.10
5	97.50	125.10	137.00
6	97.00	127.00	141.00
7	96.50	126.00	141.20
8	100.00	124.50	133.50
Average	98.50	126.07	137.55
SD	2.70	1.41	3.33
%RSD	2.74	1.10	2.40

**Table III.** The Measurement Result on the Level of Wax Content in Cooking Oil Before and After Adding the Flour

Wax Concentration ( % )	Measured level of wax content (%)	
	Before Adding the Flour	After Adding the Flour
5	5.27	4.58
5	4.92	4.75
5	4.92	4.84

The precision test was conducted in order to find out whether the developed method will still get the similar result if it is repeated. The determination of this test was done on self-made *gorengan* which is fried in hot cooking oil containing wax concentration of 0%, 5% and 10%, and then, the discoloration was observed by using univariate analysis on Photometrix<sup>®</sup> application. The measured pixel value on *gorengan* with no wax content shows a range of values from 95 to 102.5 pixels. This means that wax concentration measured on K1 quadrant shows 0% of wax concentration or less than the detection limit. The measured pixel value on *gorengan* with 5% and 10% of wax concentration are on a range of 124-128 pixels and 133-141 pixels which shows quadrant of K2 and K3. The %RSD obtained in 0%, 5% and 10% of wax concentration is 1.1% ; 2.4% showing that the analytical method has good degree of precision (Table II).

Selectivity is determined by comparing between cooking oil containing 5% of wax and fried dough. The flour selected as the matrix is potential to interfere the analysis result because flour is generally used in

making *gorengan*. The measurement result is presented in Table III. The result of statistical data processing shows that the data is normally distributed with a significance value of 0.143. The result of one-way ANOVA analysis with 5% of  $\alpha$  presents that the flour does not significantly show different result with p-value of 0.99. It can be concluded that adding the flour as matrix cannot significantly interfere the analysis. The developed method is selective enough to identify wax content in *gorengan* dough.

#### Application Method on *Gorengan* Sample

Implementation of multivariate analysis on Photometrix<sup>®</sup> application on smartphone is used to detect wax content on five different *gorengan* namely fried tofu, fried *tempe*, fried banana, fried sweet potato and *bakwan* (vegetables fritter snack). The *gorengan* samples were randomly picked in the market. This examination aims to identify the classifications among test samples. If the test sample containing wax, it will be classified on the similar quadrant. From the five samples that had been analyzed, the sample of fried *tempe* shows 5% and 10% of wax

concentration which is indicated on quadrants of K2 and K3 (Figure 3). The test result is in accordance with the research conducted by Helfer and other researchers who provide the ability of Photometrix<sup>®</sup> application in grouping banknotes based on their color from different countries based on the paper used (Helfer *et al.* 2017). As a comparative method, TLC test was conducted and it showed that there were spots on the sample of fried *tempe* with Rf value of 0.52 and 0.50 which is equivalent to Rf value of the sample of cooking oil which is deliberately added wax. This Rf value is almost similar with the Rf value of wax sample analyzed by the similar TLC system. Therefore, it can be ascertained that the spots appearing in the sample are wax spots. While the samples other than the sample of fried *tempe* do not show spots which indicate that the sample tested does not contain wax. The result shows that smartphone can be used to detect wax content on *gorengan*. Smartphone analysis method using Photometrix<sup>®</sup> application needs further development on the reagent and color image data processing in order to be used to detect wax content on lower concentration.

## CONCLUSION

The wax content on *gorengan* can be detected by using Photometrix<sup>®</sup> application on smartphone. Parameter performance of the developed method shows good result with the correlation coefficient value ( $r$ ) of 0.9537 and it is accurate with % RSD value less than 3%. This can be implemented to detect the wax content on *gorengan*.

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## THE DIFFERENCE IN THE TOTAL CHOLESTEROL/HDL-C RATIO IN TYPE-2 DM PATIENTS WITH DIABETIC ULCERS AND WITHOUT DIABETIC ULCERS IN BETHESDA HOSPITAL YOGYAKARTA

### PERBEDAAN RASIO KOLESTEROL TOTAL/HDL-C PASIEN DM TIPE 2 DENGAN ULKUS DAN TANPA ULKUS DIABETIKUM DI RUMAH SAKIT BETHESDA YOGYAKARTA

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#### ABSTRACT

*Diabetes mellitus (DM) is one of the diseases that annually shows an increase. An increase of DM prevalence will follow the increasing diabetic ulcers complication. Cholesterol could influence to create ulcer through atherosclerosis process. Ratio of total cholesterol/HDL is an important predictor to detect early risk of vascular complication in type 2 DM patients that can manifest as diabetic ulcers. This study aims to know whether there is any difference in the Total Cholesterol/HDL-C Ratio in type-2 DM patients with diabetic ulcers and without diabetic ulcers. This study was an analytic observational with cross-sectional approach. Data collection was done using the purposive sampling with a sample size of 30 patients of diabetes mellitus with diabetic ulcers and 30 patients without diabetic ulcers. The data were analyzed by Mann-Whitney. The median results in Total cholesterol/HDL-C ratio between Type-2 DM patients with diabetic ulcers and without diabetic ulcers were 6.025 and 3.680 respectively. The median of cholesterol total/HDL-C ratio Type-2 DM patients with diabetic ulcers was higher than those without diabetic ulcers. Mann-Whitney showed the value of  $P = 0.000$ , so that this study found a significant difference. In conclusion, the ratio of total cholesterol/HDL-C in type-2 DM patients with diabetic ulcer was significantly higher than type-2 DM patients without diabetic ulcers in Bethesda Hospital, Yogyakarta.*

**Keywords:** *diabetic ulcers, HDL, total cholesterol, type-2 diabetes mellitus*

#### ABSTRAK

*Diabetes melitus (DM) merupakan salah satu penyakit yang setiap tahun menunjukkan adanya peningkatan. Peningkatan prevalensi DM tentunya akan diikuti dengan peningkatan komplikasi ulkus diabetikum. Kolesterol dapat berpengaruh dalam terjadinya ulkus diabetikum melalui proses aterosklerosis. HDL memiliki peran penting dalam mencegah aterosklerosis. Rasio kolesterol total terhadap HDL merupakan prediktor yang penting untuk mendeteksi dini risiko komplikasi vaskular yang dapat bermanifestasi menjadi ulkus. Penelitian ini bertujuan untuk mengetahui apakah terdapat perbedaan rasio kolesterol total terhadap HDL pada pasien DM tipe 2 dengan ulkus diabetikum dan pasien DM tipe 2 tanpa ulkus diabetikum di RS Bethesda*



Yogyakarta. Penelitian ini merupakan penelitian observasional analitik dengan pendekatan cross sectional. Pengambilan data menggunakan purposive sampling dengan besar sampel sebanyak 30 pasien DM dengan ulkus diabetikum dan 30 pasien DM tanpa ulkus diabetikum. Data dianalisis dengan uji mann-whitney. Median Rasio Kolesterol total/HDL antara pasien DM tipe 2 dengan ulkus diabetikum dan tanpa ulkus diabetikum sebesar 6,025 dan 3,680. Pasien DM tipe 2 dengan ulkus memiliki rasio kolesterol/hdl lebih tinggi dibanding tanpa ulkus. Uji Mann-Whitney menunjukkan nilai  $p = 0,000$  sehingga penelitian ini didapatkan perbedaan yang bermakna. Kesimpulannya adalah, rasio kolesterol total/HDL pada penderita DM tipe 2 dengan ulkus lebih tinggi secara bermakna dibandingkan dengan penderita DM tipe 2 tanpa ulkus diabetikum di RS. Bethesda Yogyakarta.

**Kata kunci:** ulkus diabetikum, HDL, kolesterol total, diabetes mellitus tipe 2

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## INTRODUCTION

Diabetes mellitus (DM) is one of the diseases that shows an increasing trend annually. International Diabetes Federation (IDF 2014) stated that the number of DM patients of 366 million in 2011 increased to 387 million in 2014. Similar conditions also occur in Indonesia, according to the Basic Health Research Report (Kementrian Kesehatan Republik Indonesia, 2013), the prevalence of DM patients in 2013 (2.1%) increased from 1.1% in 2007 with the highest incidence rate in Yogyakarta Province (2.6%).

Increased incidence rates of DM will definitely be followed by an increased risk of long-term complications, which are divided into micro vascular and macro vascular. Micro vascular complications may include retinopathy, nephropathy, and neuropathy, caused by capillary and small blood vessel damage. Macro vascular complications are an illustration of the increased risk of atherosclerosis formation, in the form of coronary artery disease (CAD), cerebrovascular injury (CVA), and peripheral vascular disease (PAD) (Cagliero, 2016).

One of the most common complications of diabetes is diabetic ulcers. Diabetic ulcers are partial damage (partial thickness) or full damage (full thickness) of the skin that can extend into tissues under the skin, tendons, muscles, bones and joints that occur in the legs of a person with diabetes mellitus (Tarwoto, 2012). The prevalence of diabetic ulcer patients in Indonesia is around 15%. The case of diabetic ulcers is often the result of late

manifestations of peripheral neuropathy, Peripheral Arterial Disease (PAD) or a combination of both (Brownrigg et al., 2012).

Diabetic ulcers are often closely related to one of the macro vascular complications of DM, namely Peripheral Arterial Disease (PAD) (Rodrigues and Mitta, 2011). Peripheral arterial disease (PAD) is a condition of blockage of blood flow in peripheral blood vessels that can be caused by the process of atherosclerosis (American Diabetes Association, 2015). The blockage may cause oxygenation to the peripheral part, causing ischemia resulting in an ulcer on the foot of a DM patient, and the prolonged wound healing in the lower limb (Jeffrey et al., 2016).

The process of atherosclerosis in a DM ulcer is similar to that of a coronary heart disease. The process of atherosclerosis may occur due to lipid fraction abnormalities, such as decreased HDL, or increased total cholesterol, LDL, and triglycerides (Perkumpulan Endokrinologi Indonesia, 2015). High density of lipoprotein (HDL) plays an important role in reducing the risk of atherosclerosis because it has antiatherogenic processes, whereas total cholesterol plays a role in increasing the process of atherosclerosis because it has an atherogenic process (Jesus et al 2009).

The ratio of total cholesterol / HDL cholesterol is obtained by dividing the total amount of cholesterol by HDL. The ratio of total cholesterol / HDL cholesterol according to NCEP ATP III should be  $<4.5$  in men and

<4.0 in women. The higher the level, the more it will accelerate the process of atherosclerosis, when the blood vessels of the extremities are affected, causing the occlusion of blood vessels and ischemic that may lead to ulcers.

Based on the description, this study is important to know whether there is a difference in the ratio of total cholesterol / HDL between type-2 DM patients who have foot ulcer complications and type-2 DM patients without diabetic foot ulcer complications.

## METHODS

### Research Design and Subject

The type of this research is an observational analytical research with cross-sectional approach. The data collection using secondary data in the form of medical record employed a purposive sampling technique with a large sample of 30 DM patients with diabetic foot ulcer complication and 30 DM patients without diabetic foot ulcer complication at Bethesda hospital, Yogyakarta. The inclusion criteria of this study were all type-2 DM patients with ulcers and without ulcers who have total cholesterol and HDL data. The exclusion criterion for this study is a medical record that has incomplete data.

Data processing was done at the Center for CE & BU Study using IBM SPSS 22 program. Normality test was done first using the Shapiro-Wilk test. The difference in the ratio of total cholesterol / HDL in type-2 DM patients with ulcers and without ulcers was analyzed using the Mann-Whitney test.

## RESULTS AND DISCUSSION

The subjects in this study were 30 type-2 DM patients with ulcer complications, and 30 type-2 DM patients without ulcer complications, having characteristic features as shown in Table I.

In the comparative tests, the total cholesterol, HDL cholesterol, and the ratio between them among the 30 type-2 DM patients with ulcers and 30 type 2 DM patients

without ulcers will be compared. Table II shows the median of the total desired cholesterol levels according to NCEP ATP (<200 mg / dl) for the type-2 DM patients with ulcers and those without ulcers. The total cholesterol levels in type-2 DM patients with ulcers were higher than those without ulcer, i.e. 183.35 mg / dl and 177.50 mg / dl, but the difference was not significant because  $p = 0.679$  ( $p > \alpha$ ). This is similar to the retrospective study conducted in Saudi Arabia by Manda et al., 2012, which found higher total cholesterol levels in DM patients with ulcers, i.e. 189.89 mg / dl, compared to those without ulcers, i.e. 175.75 mg / dl and both levels were categorized as desirable according to NCEP ATP III (<200 mg / dl), although the results were different although insignificant.

The results of this study also showed a significant decrease ( $p = 0.000$ ) of the High Density Lipoprotein (HDL) levels in type-2 DM patients with ulcers, as shown in Table II that the median of the HDL values was about 29.65 mg / dl, whereas the median in type-2 diabetes mellitus patients without ulcers was higher, at about 48.00 mg / dl. The results of this study are similar to the prospective cohort studies conducted in India by Mohammad, et al. (2012) on 162 DM patients without ulcers and 162 DM patients with ulcers. The study found a decrease in HDL in DM patients with ulcers, with the mean of HDL values at around 34.6 mg / dl. The DM patients without ulcers had a higher mean of HDL at about 44.3 mg / dl, with a significant difference of ( $p = 0.005$ ).

Although the median of the total cholesterol levels of the type-2 DM patients with ulcers and without ulcers was included in the desired category (<200 mg / dl) according to NCEP-ATP III, it would remain a high risk if the HDL levels decreased from the normal level, since the ratio of total cholesterol to HDL increased. In the study conducted by Dionyssiou et al., in Greece in 2002, the total cholesterol did not differ significantly, and only a significant decrease in HDL could increase the ratio between the two in DM patients with lower limb occlusive disease caused by the atherosclerosis process.

**Table I.** Basic Characteristics of the Research Subjects Based on the Diabetic Ulcers and Non-Diabetic Ulcers

Characteristics	Type-2 DM patients with ulcers	Type-2 DM patients without ulcers
Sex (%)		
Male	13 (43.3%)	5 (16.7%)
Female	17 (56.7%)	25 (83.3%)
Age (Year)	55.50 (28-77)*	55.50 (35-86)*
> 50 years old (%)	22 (73.33%)	23 (76.67%)
< 50 years old (%)	8 (26.67)	7 (23.33%)
Total Cholesterol (mg/dL)	183.35 (79.7-328.2)*	177.50 (96.7-260.3)*
High	4 (13.33 %)	8 (26.67 %)
Borderline	8 (26.67 %)	3 (10%)
Desired	18 (60 %)	19 (63.33%)
HDL (mg/dL)	29.65 (5.60-49.50)*	48.00 (33.20-69.70)*
Ratio of Total Cholesterol /HDL (mg/dL)	6.025 (3.44-43.30)*	3.680 (2.20-5.45)*
Abnormal LDL (%)	13 (43.33%)	22 (73.33%)
Hypertriglyceride (%)	16 (53.33%)	8 (26.67)
Pre-prandial blood sugar level (mg/dL)	228 (122-322)*	176.65 (74.10-351.00)*
Random blood glucose level(mg/dL)	262.0 (137.40-675.0)*	222.0 (79.00-414.0)*
blood glucose levels 2 hours post prandial (mg/dL)	265.50 (87.00-513.60)*	227.35 (84.00-488.70)*
Hypertension (%)	20 (66.67%)	15 (50%)

Notes: \*the data is presented in median (minimum-maximum) because it was not normally distributed

**Tabel II.** Mann-Whitney Statistical Test on Type-2 DM Patients with Ulcers and Without Ulcers

Characteristics	Type-2 DM		p-value
	Ulcer (n=30)	Non-Ulcer (n=30)	
Total Cholesterol (mg/dL)	183.35 (79.70-328.20)	177.50 (96.70-260.30)	0.679
HDL (mg/dL)	29.65 (5.60-49.50)	48.00 (33.20-69.70)	0.000
Ratio of Total Cholesterol/HDL (mg/dL)	6.025 (3.44-43.30)	3.680 (2.20-5.45)	0.000

HDL and total cholesterol cannot be self-assessed based merely on the high or normal classification of cholesterol levels. Both must be assessed to get the ratio value. The National Cholesterol Education Program (NCEP, 2001) also recommends that total cholesterol measurement be included in HDL measurements (Chen et al., 2016). The effect of decreased HDL cholesterol levels on the risk of atherosclerosis will be greater than the increase in total cholesterol (Ikura et al., 2015).

The total acceptable cholesterol/ HDL ratio according to NCEP ATP III is <4.5 mg /

dl for men and <4 mg / dl for women. In Table II, the results of this study found a significant difference in total cholesterol / HDL ratio (p = 0.000) between type-2 DM patients with ulcer and those without ulcers. The median in the ratio of total cholesterol / HDL of type 2 DM patients with ulcers was 6.025 mg/dL, whereas patients without ulcers had an acceptable ratio median of about 3.680 mg/dL.

A variety of simple indices involving cholesterol, the ratio of total cholesterol / HDL known as the Castelli I index is the strongest predictor for the risk of vascular complications, as it reflects the overall balance

between atherogenic and antiatherogenic (Jesus et al., 2009). This finding is corroborated by the research of Jiang et al., 2004, which, after 6 years of monitoring, found that the ratio of total cholesterol / HDL was the best predictor of vascular complications compared to LDL or other lipid profiles in type-2 DM patients. Cross-sectional studies, conducted by Olamoyegun et al., 2016 in Nigeria with 699 subjects, also proved that the ratio of total cholesterol / HDL (the Castelli I index) was a better predictor of vascular complications than other lipids, with the value of ( $p < 0.001$ ).

Peripheral arterial disease (PAD) and peripheral vascular disease are the most common processes of diabetic ulcers, due to prolonged duration of wound healing and amputations in DM patients (Rodrigues and Mitta, 2011). The high ratio of total cholesterol / HDL affects the incidence of diabetic ulcers via PAD resulting from the process of atherosclerosis, with a mechanism similar to the formation of atherosclerosis in cardiovascular disease. The cohort study, conducted by Mounier et al., in 2007, found a significant increase in the ratio of total cholesterol / HDL in the PAD study subjects. It proved the importance of the role of the Castelli I index (total cholesterol / HDL) in PAD incidence caused by atherosclerosis.

A study was conducted in Taiwan by Te et al., in 2007, to monitor the type-2 DM patients for 3 years. The ratio of total cholesterol / HDL was also the most important independent predictor for changes in the value of ABI (Anckle Brachial Index), the high ratio of total cholesterol / HDL can predict a decrease in brachial ankle index in the subject of type-2 Asian DM patients. It highlights the role of the ratio of total cholesterol / HDL to decrease blood circulation in the extremities.

Although the results of the total cholesterol levels are optimal, but if the HDL is low, the ratio between the two will increase and the risk of atherosclerosis increases, considering that HDL has anti-atherosclerotic effect. Part of the atheroprotective effect is associated with the transport of excess cholesterol deposited in the blood vessels to

the liver (Bleda et al., 2012). The effects of antioxidants inhibit the oxidation of cholesterol in the blood vessels. HDL also plays a role in protecting endothelial function, which increases NO (nitric oxide) which plays a role in vasodilation, and reduces expression of intracellular adhesion molecule (ICAM) -1 and E-selection, an IL-8 cytokine that increases the binding of leukocytes to atheroma formation. Endothelial apoptosis is also known to be prevented by HDL (Ahn and Kim, 2016).

Low HDL levels will cause a decrease in endothelial protection, resulting in endothelial dysfunction, accumulation of cholesterol in the blood vessels; and causing atherosclerosis. If the formation of atherosclerosis occurs in peripheral blood vessels (in this case lower extremities), it will cause PAD, marked by blood vessel occlusion, and will cause stenosis, a condition where blood and oxygen supply to tissues in the lower extremity will be impaired, marked by the loss or decrease in pulse in the dorsal pedic, tibial, and popliteal arteries. The patient's legs experience atrophy and coldness; and nails thicken due to ischemic, resulting in the condition of ulcers in the foot that usually starts from the tip of the toe and the ulcer healing process will be disrupted if oxygen supply remains inadequate (Schaper et al., 2012).

Increased incidence of diabetes mellitus is usually followed by the complication of diabetic mellitus ulcers in the community. Therefore, it is important to assess and evaluate the risk factors. It is possible to improve early prevention strategies and early risk assessments. Regular lipid level checks can be performed to prevent undesirable conditions. The examination of total cholesterol should always be accompanied by HDL examination, to control the ratio between the two cholesterol levels. This is to detect earlier the ratio of total cholesterol to abnormal HDL which is a risk factor of type-2 DM complications, in this case diabetic ulcers. This research should still be developed to obtain more valid and tested data. Development of this research can be done by

using primary data samples or using other more accurate research methods.

The limitation of this study is that it does not consider other factors that can interfere with the results. These factors are the patients' duration in suffering from DM, HbA1c levels as the sign of controlled diabetes, use of fat-lowering drugs, menopause-related sex factors, socio-demographic and economic factors, as these factors may affect the results of the study. Lipid levels are strongly influenced by lifestyle, diet, level of patients' education in the diet and foot care recommended by doctors and health workers who are responsible for the patients' diets.

## CONCLUSION

There was a significant difference of in the total cholesterol / HDL ratio between type-2 DM patients with diabetic ulcers and type-2 DM patients without diabetic ulcers in Bethesda Hospital. The ratio of the total cholesterol / HDL in type-2 DM patients with diabetic ulcers is significantly higher than type-2 DM patients without diabetic ulcers.

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## HEPATOPROTECTIVE EFFECT OF FOREST HONEY ON CARBON TETRACHLORIDE INDUCED FEMALE WISTAR RATS

### EFEK HEPATOPROTEKTIF MADU HUTAN PADA TIKUS BETINA GALUR WISTAR YANG DIINDUKSI KARBON TETRAKLORIDA

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#### ABSTRACT

Hepatoprotective effect study of forest honey had been conducted on a female rat induced with carbon tetrachloride (CCl<sub>4</sub>). The study aimed at obtaining the scientific data and the evidence of forest honey as hepatoprotective agent on the rat. The study was a true experimental study with a single factor completely randomized design. Thirty rats were randomly divided into six groups (n=5). Group I received carbon tetrachloride 2.0 mL/kgBW intraperitoneally, group II received olive oil 2.0 mL/kgBW intraperitoneally, group III received forest honey 8.1 mL/kgBW (6 days, peroral), groups IV, V, VI were given forest honey 3.6, 5.4, 8.1 mL/kgBW (6 days, peroral) and intraperitoneal induction of carbon tetrachloride 2 mL/kgBW on seventh day. The blood sample of all rats were taken for ALT-AST measurement and their liver were sampled for histological examination of the liver cell. Groups I and III on the seventh day, group II on the second day, groups IV,V,VI on the eighth day. The result showed that a forest honey can be used as a hepatoprotective agent on the female rat Wistar strain induced by carbon tetrachloride 2 mL/kgBW with doses 3.6, 5.4, 8.1 mL/kgBW.

**Keywords:** ALT-AST, carbon tetrachloride, forest honey, liver histopathology

#### ABSTRAK

Telah dilakukan penelitian efek hepatoprotektif madu hutan pada tikus betina terinduksi karbon tetraklorida (CCl<sub>4</sub>) dengan tujuan membuktikan efek hepatoprotektif madu hutan pada tikus galur wistar yang diinduksi karbon tetraklorida. Jenis penelitian ini adalah eksperimental murni dengan rancangan acak lengkap pola searah. Tiga puluh ekor tikus dibagi secara acak ke dalam enam kelompok (n=5). Kelompok I diberi karbon tetraklorida 2,0 mL/kgBB secara intraperitoneal (i.p). Kelompok II diberi olive oil 2,0 mL/kg BB secara i.p. Kelompok III diberi madu hutan 8,1 mL/kgBB (6 hari, peroral (p.o)); kelompok IV, V, VI diberi madu hutan 3,6; 5,4; 8,1 mL/kgBB (6 hari, p.o) dan diinduksi secara i.p dengan karbon tetraklorida 2 mL/kgBB pada hari ketujuh. Kemudian dilakukan pengambilan sampel darah untuk diukur aktivitas serum alanin aminotransferase dan aspartat aminotransferase (ALT-AST) dan pengambilan organ hati untuk pengamatan histopatologi sel hati. Kelompok I dan III pada hari ketujuh, kelompok II pada hari kedua; kelompok IV, V, VI pada hari kedelapan. Hasil penelitian menunjukkan bahwa madu hutan memiliki efek hepatoprotektif pada tikus betina galur Wistar terinduksi 2,0 mL/kgBB karbon tetraklorida dengan dosis 3,6; 5,4; dan 8,1 mL/kgBB.

**Kata kunci:** ALT-AST, karbon tetraklorida, madu hutan, histopatologi hati

## INTRODUCTION

Liver is the central organ in human body's metabolism system and it is very important for survival, both as a protection, detoxification, and even metabolism (Price, 2015). Liver could still maintain its function despite small damage because the existence of endogenous antioxidant helps the process of liver regeneration. On the other hand, a huge damage will cause imbalance between endogenous antioxidant and radical which will cause a quite massive liver disfunction (Jamilla et al., 2017).

The etiology of chronic liver disease that often happened in Asia includes: hepatotropic virus and non-hepatotropic, hepatitis B or C reactivation, infectious agent that grows in the hepar, alcohol, the usage of hepatotoxic medication, autoimmune hepatitis (Wilson's disease) and surgery intervention, also the hepatotoxic etiology which have not been recognized yet (Sarin et al., 2009). One of the hepatotoxic material that had the potential to cause chronic liver disease was carbon tetrachloride (CCl<sub>4</sub>). Carbon tetrachloride (CCl<sub>4</sub>) was included into the list of hepatotoxic material that could produce radicals which is hidden in body fat, hepar, and backbone marrow. CCl<sub>4</sub> caused hepar damages through stress oxidative reaction and biochemistry mechanism (Monika, 2012). The process of hepar damage caused by CCl<sub>4</sub> induction could be prevented by antioxidant (Tjok and Wibawa, 2012).

Honey is one of the most popular natural resources that is frequently used as traditional medicine (Erguder et al., 2008). Honey contains fructose, glucose, and material that functions as antioxidant, like phenolic compound, chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Chen et al., 2000; Nagai et al., 2006), and flavanoid compounds such as luteolin, quercetin, apigenin, fisetin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin (Erguder et al., 2008). It could be a form of remedy to some diseases, including heart disease. According to Erguder et al. (2008), honey is useful for preventing heart damages due to bile duct obstruction. Meanwhile, based on the

research conducted by Mahesh et al. (2009), Indian honey could protect heart from oxidative damages and could be used as an effective hepatoprotector of heart damages due to paracetamol induction. In addition, research by Halawa et al. (2009) concluded that honey could modulate heart and kidneys' cell damages of a rat that was previously induced by lead. National Honey Board (2005), stated that one of the advantages of honey is that it is rich of antioxidant. Researches showed that honey is indeed a huge source of antioxidant. However, the quantity and the rate of antioxidant contained depend on the nectar's source. Darker-colored honey (e.g forest honey) is proved to have higher rate of antioxidant than lighter-colored honey (e.g. acacia honey) (Suranto, 2007). Therefore, forest honey is allegedly able to prevent heart damages. Up until this moment, there is still no research about the potential of forest honey as a hepatoprotection agent.

## METHODS

The materials used in this research were forest honey sourced from the bees that could be found in Sialang trees located in Tesso Nilo National Park, Pelalawan, Riau, Sumatera. While the experimental animals were galur female Wistar rat weighed 100-200 grams and aged 1-2 months old collected from Hayati Imono Laboratory of the Faculty of Pharmacy Sanata Dharma University, Yogyakarta. Chemicals used were carbon tetrachloride, olive oil, aquadest, aquabidest, NaCl 0,9%, formaldehyde 10%, ALT and AST assay kits. The instruments used were beaker, measuring cylinder, volumetric flask, stirring rod, analytical scale, spoon, pipette, centrifuge, vortex, p.o and i.p syringes, hematocrite, Eppendorf<sup>®</sup> cylinder, tweezers, scalpel, ointment pots, Microlab<sup>®</sup>, and microscope.

The research protocol and procedures taken had attained approval from the Medical and Health Research Ethics Committee (MHREC) of Gajah Mada University's Faculty of Medicines. Thirty (30) experimental animals were randomly distributed into 6 treatment groups that each consisted of 5 experimental animals. Group I



was given 2.0 mL/kgBW of carbon tetrachloride material based on the i.p. Group II was given 2.0 mL/kgBW olive oil based on the i.p. Group I and II's blood withdrawal was done after 24 hours. Group III was given forest honey with the dosage of 8.1 mL/kgBW for six consecutive days based on p.o and the blood withdrawal was done on the seventh day. Group IV to VI were given forest honey (in order) 3.6; 5.4; 8.1 mL/kgBW based on p.o once a day for six consecutive days. And on the seventh day, the groups were given carbon tetrachloride 2mL/kgBW. Twenty four hours after carbon tetrachloride induction, ±2 mL of blood was withdrawn through retro-orbital sinus method. Then, the activities of ALT and AST serums were measured (Utomo, 2015). Next, the rat was sacrificed as its liver was taken with the purpose of making its hepatology glass microscope slides. The ALT and AST serums' activities were analyzed using Saphiro-Wilk and continued by One Way ANOVA test with the confidence level of 95%, and Post Hoc LSD test to discover the differences between each group. Hepar's hepatology examination's result was analyzed descriptively to find out the existence of possible damages and refinements on the cell.

**RESULTS AND DISCUSSION**

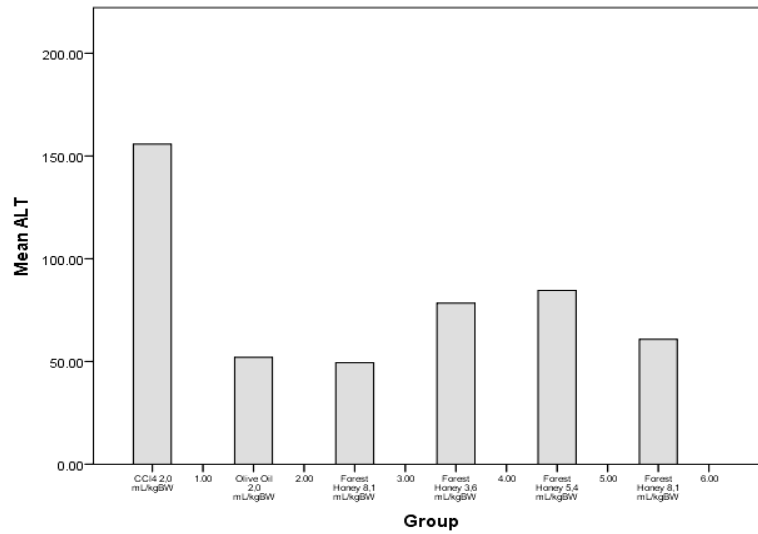
ALT and AST activities on the three dosages of treatment 3.6; 5.4; and 8.1 mL/kgBW and CCl<sub>4</sub> control group, olive oil control's data could be seen on Table I.

The research result showed that ALT serum's activities of forest honey treatment of 3.6; 5.4; and 8.1 mL/kgBW were consecutively 78.40±20.54; 84.60±15.14; and 60.80±8.14 U/L (Figure 1). Based on *Post Hoc* LSD Test, the average of all groups' ALT activities of forest honey treatment was significantly different from 155.80±18.31 U/L (p<0,05) carbon tetrachloride control and insignificantly different from 52.00±2.07 U/L (p<0.05) olive oil control. This result proved that 3.6; 5.4; and 8.1 mL/kgBW of forest honey could lower the activity level of ALT serums to the normal range. Based on the hepatoprotective percentage (Table I) of 3.6; 5.4; and 8.1 mL/kgBW of forest honey consecutively scored 74.57%; 68.59% and 91.52%. This showed that hepatoprotective percentage possessed by the three forest honey treatment groups resulted near to the number of total healing which is 100%.

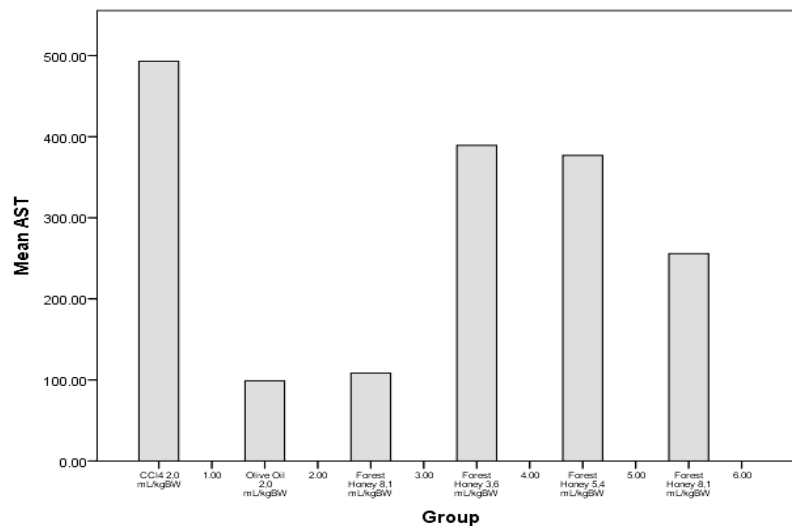
**Table I.** The Effect of Applying Forest Honey on ALT and AST Serums' Activities of Carbon Tetrachloride-Induced Rats

Groups	ALT ± SE Averages (U/L)	AST ± SE Averages (U/L)	Hepatoprotective %	
			ALT	AST
I	155.80 ± 18.31 <sup>b,c,1,2,3</sup>	493.00 ± 29.97 <sup>b,c,1,2,3</sup>	-	-
II	52.00 ± 2.07 <sup>a</sup>	99.00 ± 4.85 <sup>a,1,2,3</sup>	-	-
III	49.40 ± 6.25 <sup>a</sup>	108.40 ± 9.71 <sup>a,1,2,3</sup>	-	-
IV	78.40 ± 20.54 <sup>a</sup>	389.40 ± 70.38 <sup>a,b,c,3</sup>	74.57%	26.29%
V	84.60 ± 15.14 <sup>a</sup>	377.00 ± 16.49 <sup>a,b,c,3</sup>	68.59%	29.44%
VI	60.80 ± 8.14 <sup>a</sup>	255.80 ± 23.33 <sup>a,b,c,1,2</sup>	91.52%	60.20%

Notes: I: 2.0 mL/kgBW CCl<sub>4</sub> control; II: 2.0 mL/kgBW olive oil control; III: 8.1 mL/kgBW forest honey control; IV: 3.6 mL/kgBW forest honey + 2.0 mL/kgBW CCl<sub>4</sub>; V: 5.4 mL/kgBW forest honey + 2.0 mL/kgBW CCl<sub>4</sub>; VI : 8.1 mL/kgBW forest honey + 2.0 mL/kgBW CCl<sub>4</sub>; SE=standard error; a=significantly different (p<0.05) towards carbon tetrachloride control; b=significantly different (p<0.05) towards olive oil control; c=significantly different (p<0.05) towards forest honey control; 1,2 and 3=significantly different (p<0.05) towards forest honey 3.6; 5.4 and 8.1 mL/kgBW



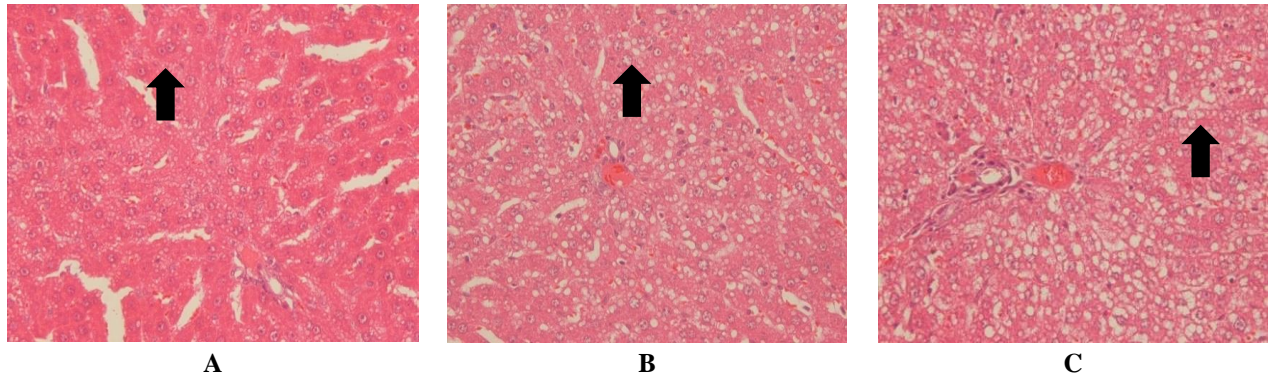
**Figure 1.** The effect of applying forest honey on ALT serum’s activities of carbon tetrachloride-induced rats diagram



**Figure 2.** The effect of applying forest honey on AST serum’s activities of carbon tetrachloride-induced rats diagram

AST serum’s activities of forest honey treatment 3.6; 5.4; and 8.1 mL/kgBW scored consecutively  $389.40 \pm 70.38$ ;  $377.00 \pm 16.49$ ; and  $255.80 \pm 23.33$  U/L (Figure 2). Based on *Post Hoc* LSD Test, the average of all groups’ AST serum activities of forest honey treatment was significantly different from  $493.00 \pm 29.97$  U/L ( $p < 0.05$ ) of carbon tetrachloride control and from  $99.00 \pm 4.85$  U/L ( $p < 0.05$ ) of olive oil control. This result proved that 3.6; 5.4; and 8.1 mL/kgBW of forest honey could lower the activity level of AST serums but still have not succeeded to decrease the number into normal range. Based on the hepatoprotective

percentage (Table I) of 3.6; 5.4; and 8.1 mL/kgBW of forest honey consecutively scored 26.29%; 29.44% and 60.20%. This showed that the hepatoprotective percentage possessed by the three forest honey treatment groups still have not reached close to the percentage of total healing which is 100%. It was strengthened by a microscopic imagery of the rats’ hepatocyte (Figure 3.a, b and c) which could be seen that on every forest honey treatment group, degenerative fattening still happened on the periportal space and around the veins on its hepatocyte cells.



**Figure 3.** Microscopic imageries of degenerative fattening on a rat's hepatocyte after applying forest honey of 3.6 mL/kgBW (A), 5.4 mL/kgBW (B), and 8.1 mL/kgBW (C).

The high-level AST serum's activities was caused by the existing damages on other organs such as the heart, muscles, and kidneys considering that AST is not specifically located in hepar only (Thapa and Anuj, 2007). Hepar is one of the most sensitive organs to exposures of carbon tetrachloride. However, it is still probable that carbon tetrachloride that was induced systematically based on i.p could cause damages on heart, kidneys, muscles, brain, and lungs.

According to the research, 3.6; 5.4; and 8.1 mL/kgBW forest honey had the hepatoprotective effects since it could lower ALT and AST serums' activities level of carbon tetrachloride induced rats until it was significantly different from carbon tetrachloride. Honey contains fructose, glucose, and compounds which function as antioxidant, like phenolic compound, chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Chen et al., 2000; Nagai et al., 2006), and flavanoid compounds such as luteolin, quercetin, apigenin, fisetin, kaempferol, isorhamnetin, acacetin, tamarixetin, chrysin, and galangin (Erguder et al., 2008). Kandimalla (2006) stated that flavanoid has the ability to serve as a hepatoprotective that could lower enzim (SGPT, SGOT, ALP, and LDH) serums on carbon tetrachloride induced rats. Flavanoid has a high electronegativity level which enables it to neutralize radicals by contributing hydrogen atoms to free electrons of  $CCl_3^-$  and  $CCl_3OO^-$ . It means that it is able to prevent

any possible bonding between radicals and fatty acid located in hepar so that hepar damages can be resolved.

### CONCLUSION

Forest honey on dosages of 3.6; 5.4; and 8.1 mL/kgBW possessed the hepatoprotective effect on 2.0 mL/kgBW of a carbon tetrachloride induced female rat Wistar strain.

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## THE RELATIONSHIP BETWEEN PHYSICAL ACTIVITY AND OBESITY BASED ON BODY FAT PERCENTAGE IN BANJAROYO VILLAGE, KALIBAWANG, KULON PROGO, D.I. YOGYAKARTA

### HUBUNGAN AKTIVITAS FISIK TERHADAP KEJADIAN OBESITAS BERDASARKAN BODY FAT PERCENTAGE DI DESA BANJAROYO, KALIBAWANG, KULON PROGO, D.I. YOGYAKARTA

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#### ABSTRACT

*Obesity, a metabolic disorder characterized by the accumulation of excessive body fat, which is closely associated with metabolic derangement-related disease. Obesity can be caused by low physical activity that can be assessed using body fat percentage. The purpose of this study was to identify the relationship between physical activity and obesity based on body fat percentage in Banjaroyo village, Kalibawang, Kulon Progo, Special Region of Yogyakarta. This study was an observational-analytical study with cross sectional design and conducted in January-June 2017. Total samples were 243 people (86 males and 157 females) with age range between 18-65 years old. Physical activity data were collected using a structured interview based on International Physical Activity Questionnaire (IPAQ), while body fat percentage was taken using Bioelectrical Impedance Analysis (BIA). The data were analyzed using comparative Chi-square test with the level of significance of 95%. The prevalence of obesity based on body fat percentage was 78.2% and 21.8% in men and women, respectively. Results of the study showed that there is no significant relationship between physical activity and obesity based on body fat percentage ( $p=0.419$ ; CI 95%: 0.66-2.689). In conclusion, there is no significant relationship between physical activity and incidence of obesity.*

**Keywords:** *body fat percentage, physical activity, obesity*

#### ABSTRAK

*Obesitas merupakan penyakit metabolik yang ditandai dengan akumulasi lemak yang berlebihan. Aktivitas fisik yang rendah menjadi salah satu penyebab terjadinya obesitas. Keadaan obesitas dapat diukur dengan menggunakan persentase lemak tubuh yang didefinisikan sebagai proporsi dari massa lemak tubuh seseorang. Tujuan dari penelitian ini adalah untuk mengidentifikasi hubungan antara aktivitas fisik dengan kejadian obesitas berdasarkan Body Fat Percentage (BFP) pada warga di Desa Banjaroyo, Kalibawang, Kulon Progo, D.I.Yogyakarta. Jenis penelitian yang dilakukan adalah observasional analitik dengan rancangan penelitian potong lintang dan dilakukan pada bulan Januari- Juni 2017. Jumlah sampel penelitian sebanyak 243 responden yang terdiri dari 86 pria dan 157 wanita dewasa dengan rentang usia 18 – 65 tahun. Aktivitas fisik dinilai melalui panduan wawancara International Physical Activity*

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*Questionnaire (IPAQ) dan body fat percentage dinilai dengan instrumen Bioelectrical Impedance Analysis (BIA). Analisis statistik dilakukan dengan uji komparatif Chi-square dengan taraf kepercayaan 95%. Prevalensi obesitas berdasarkan body fat percentage adalah 78,2% pada wanita dan 21,8% pada pria. Hasil penelitian menunjukkan tidak terdapat hubungan yang bermakna antara aktivitas fisik terhadap obesitas berdasarkan body fat percentage ( $p=0,419$ ; CI 95%: 0,66-2,689). Kesimpulan penelitian ini adalah tidak ada hubungan bermakna antara aktivitas fisik dan kejadian obesitas.*

**Kata kunci:** *body fat percentage, aktivitas fisik, obesitas*

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## INTRODUCTION

Obesity is a metabolic disease characterized by excessive accumulation of fat that can lead to some diseases; cardiovascular, diabetes, and joint disease (Zeng et al., 2012). As many as 600 million people around the world (13%) suffer from obesity in 2014. Obesity causes more deaths than underweight (WHO, 2016). In Indonesia, the prevalence of obesity among adults is 14.76%. In 2013, prevalence of obesity was 19.7% in men and 32.9% in women. Prevalence of obesity of men and women in 2013 has increased, compared to 2007 (13.9% in men and 13.9% in women) and 2010 (7.8% in men and 15.5% in women). Prevalence of obesity among women has increased from 2007 (18.1%) and from 2010 (15.5%) (Kementrian Kesehatan Republik Indonesia, 2013). The Province of Special Region of Yogyakarta is one of the 16 provinces with the highest prevalence of obesity in the national scale (Kementrian Kesehatan Republik Indonesia, 2016).

Regular physical activity is useful to control weight and prevent chronic diseases that can occur due to the obesity (Kementrian Kesehatan Republik Indonesia, 2011). Adequate physical activity for adults can reduce the risk of hypertension, coronary heart disease, stroke, diabetes and cancer (Widiantini and Tafal, 2014). Percentage of the lack of physical activity in rural area is 54.03%. This can cause obesity which is 13.36% higher than society with adequate physical activity (Sudikno et al., 2010). According to research conducted by Sunu et al. (2017), 42.2% of communities in Cangkringan, Sleman Regency, Yogyakarta, are obese. The obesity percentage is higher in

women (32.4%) than in men (9.8%). Transportation and informatics technology in rural area has influenced most of the communities less engaged in physical activity (Lita, 2016).

Anthropometric measurement can be used to measure body fat percentage. Body fat percentage is the proportion of fat mass in human's body. Body fat percentage (BFP) has a direct significant relationship to the increase of risk factor for cardiovascular disease, such as total cholesterol, triglyceride, low-density lipoprotein cholesterol, and fasting plasma glucose. The research also shows that BFP is a predictor of more closely related cardiovascular disease than body mass index (BMI) (Zeng et al., 2012).

Method that is used to determine someone's obesity status is body mass index (BMI), bioelectrical impedance analysis (BIA), waist to hip ratio (WHR), and waist circumference (WC). Among these methods, BIA used to measure percentage of body fat is a method that has a significant relationship with the body fat composition of a person compared to other methods (Duncan and Nevill, 2010). Bioelectrical Impedance Analysis (BIA) is used to predict the total of body fat by running the technique of weak electrical current through the body (Hoeger and Hoeger, 2013). The Bioelectrical Impedance Analysis (BIA) has a principle that lean mass contains ion which is in aqueous solution can deliver electrical signal compared to fat mass (Goonasegaran et al., 2012). The use of BIA is relatively safe because it applies low electrical current with low frequency (Ramadhan and Billy, 2017).

According to the above description, this research aims to identify the relationship of physical activity toward obesity according to body fat percentage in Banjaroyo Village, Kalibawang, Kulon Progo, Special Region of Yogyakarta.

## METHODS

### Research Design and Subject

This research was analytical observational research with cross-sectional design. The research respondents were the community of Banjaroyo Village, Kalibawang, Kulon Progo, aged 18-65 years and had been willing to sign informed consent. The type of purposive sampling with non-random technique was used as the sampling technique. The inclusion criteria of this research were healthy adults, aged 18-65 years old, male and female, who did not do vigorous physical activity 12 hours before the measurement, did not consume alcohol 48 hours before the measurement and who were willing to sign the informed consent. Meanwhile, the exclusion criteria were people with physical disabilities who cannot perform physical activity, are pregnant, consume alcohol, are absent in the data collection, and are not willing to sign the informed consent. The procedures used in this research had been approved by the Medical and Health Research Ethics Committee of Medicine Faculty of *Universitas Gadjah Mada*, number: KE/FK/0837/EC/2017.

### Data Collection

The data collection was conducted by measuring height, fat percentage and physical activity using an interview method which refers to IPAQ structured interview guide. Height measurement was conducted by using stature meter with Height® which is validated in *UPT Metrology Legal* of Yogyakarta. The fat percentage measurement tool of this research was body fat scale with Omron®. The body fat scale had been validated using the secondary validation by comparing body fat percentage measured using Bioelectrical Impedance Analysis with Omron® with skinfold thickness. Skinfold thickness used to validate BIA with *pzi dou fi* brand, has been

calibrated at the Calibration Laboratory of Testing Center and Goods Quality Certification of Surakarta.

### Body Fat Percentage Measurement

The data used in Bioelectrical Impedance Analysis for measuring the value of Body Fat Percentage (BFP) were age, gender, and height. The measurement was Bioelectrical Impedance Analysis scale with upright position, facing straight forward and using no footwear and accessories (Gonzalez-Corra and Caicedo-Eraso, 2012; Citra, 2015). The respondents could go up to the scale and step on the foot electrode after the respondents' data had been entered to Bioelectrical Impedance Analysis. The respondents were required not to move until the measurement was complete (OMRON Healthcare Asia, 2016). The result of body fat percentage would be classified based on American Council on Exercise. According to American Council on Exercise (2010), someone is said to be obese if the value of body fat percentage in woman >32% while in man >25%.

### Physical Activity Assessment

There are three categories of physical activity namely vigorous, moderate, and low. The physical activity with low intensity keeps the heart rate normal, such as walking and sweeping. The physical activity with moderate intensity increases the heart rate and produces less sweat, such as walking fast, cycling, dancing and gardening. Meanwhile, the physical activity with vigorous intensity requires energy, increases the heart rate, produces more sweat when much energy is released, such as exercising, hoeing and running (Befort et al., 2012; Intercollegiate & Network, 2010; WHO, 2017).

International Physical Activity Questionnaire (IPAQ) short form can be used to categorize physical activity of adult population aged 15-65 years (IPAQ Researcher Committee, 2005). Unit of measurement for the value of physical activity is kcal/kg/hour or MET (Metabolic Equivalents). IPAQ short form consists of seven questions used to measure someone's

physical activity during the last seven days. There are three categories based on the classification of physical activity; low physical activity <600 MET-minute/week, moderate physical activity ≥600 MET-minute/week and vigorous physical activity ≥3000 MET-minute/week (IPAQ Research Committee, 2005). Physical activity data obtained from interview result then processed by using IPAQ protocol.

The interview guide used in this research had been given to the professional judgment in order to ensure that IPAQ questionnaire is a proper translation from English into Indonesian. The language comprehension test was conducted to ensure that the language used was well understood by the respondents and was conducted on 12 respondents aged 18-65 years from Duren Sawit sub-village, Banjaroyo village, Kalibawang, Kulon Progo, Special Region of Yogyakarta, who have

similar characteristic with the residents of Banjaroyo village (Notoatmodjo, 2012). Interview guideline of physical activity used by the researcher was not examined for its validity and reliability because the interview guideline IPAQ-SF questionnaire has been used by other institutions (Marcelia, 2014; Wibowo, 2014).

**Statistical Analysis**

The data collection was conducted in the Research Center of Clinical Epidemiology and Biostatistic Unit of *Universitas Gadjah Mada Yogyakarta* by using IBM SPSS 22 program. Comparative test using Chi-square was conducted on physical activity and body fat percentage. Significant test between the observed data and the expected data was conducted by using 95% of trust level (Dahlan, 2014).

**Table I.** Demographic Characteristics of Research Respondents (N=243)

Variable		Female (n=157) n (%)	Male (n=86) n (%)	Total (n=243) n (%)	p-value
Age	41-65	113(46.5)	62 (25.5)	175 (72.0)	0.984*
	18-40	44 (18.1)	24 (9.9)	68 (28.0)	
Physical Activity	Low-moderate	31 (12.8)	8 (3.3)	39 (16.0)	0.034*
	Vigorous	126 (51.9)	78 (32.1)	204 (84.0)	
BFP	Obesity	83 (34.2)	25 (10.3)	108 (44.4)	<0.001*
	Non-obesity	74 (30.5)	61 (25.1)	135 (55.6)	
Smoking habit	Not Smoking	157 (64.6)	53 (21.8)	210 (86.4)	<0.001**
	Smoking	0 (0)	33 (13.6)	33 (13.6)	
Occupation	Unemployed	58 (23.9)	7 (2.9)	65 (26.7)	<0.001*
	Underemployed	99 (40.7)	79 (32.5)	178 (73.3)	
Dietary Habits	Less	1 (0.4)	1 (0.4)	2 (0.8)	1.000**
	Enough	156 (64.6)	85 (35.0)	241 (99.2)	
Education Level	≤ Middle School	131 (53.9)	63 (25.9)	194 (79.8)	0.058*
	≥ High School	26 (10.7)	23 (9.5)	49 (20.2)	
Income	Low	116 (47.7)	42 (17.3)	158 (65.0)	<0.001*
	High	41 (16.9)	44 (18.1)	85 (35.0)	

\* Chi-square Test; \*\* Fisher Test

**Table I.** The Relationship of Physical Activity toward Obesity based on BFP in Research Respondents in Banjaroyo Village

Variable	Obesity		Non-obesity		p	OR	95% CI
	n	%	n	%			
Physical Acitivity							0.66-2.689
Low-Moderate	19	17.30	18	13.5	0.419 <sup>e</sup>	1.33	
Vigorous	91	82.70	115	86.5			

Note:

<sup>e</sup>Comparative Test of *Chi-Square* p >0.05 = does not significantly influence



**Table III.** Others Factors influencing Obesity in Banjaroyo Village (N=243)

Variable	Obesity		Non-Obesity		p-value	OR (95% CI)
	n	%	n	%		
Gender						
Female	83	34.2	74	30.5	<0.001 <sup>f*</sup>	2.737 (1.562-4.769)
Male	25	10.3	61	25.1		
Age						
41-65 years old	84	34.6	91	37.4	0.074*	1.692(0.948-3.020)
18-40 years old	24	9.9	44	18.1		
Dietary Habits						
Less	1	0.4	1	0.4	1.000**	1.252 (0.077-20.256)
Enough	107	44	134	55		
Smoking Habits						
Not Smoking	99	40.7	111	45.7	0.033 <sup>f**</sup>	2.378(1.055-5.360)
Smoking	9	3.7	24	9.9		

Note:

<sup>f</sup>Comparative Test of *Chi-Square* p <0.05 = does significantly influence

\*Uji *Chi-square*; \*\*Uji *Fisher*

## RESULTS AND DISCUSSION

There were 243 respondents consisting of 157 female respondents and 86 male respondents fulfilling the inclusion criteria. The demographic characteristics of research respondents in Village Banjaroyo covered age, physical activity, BFP, smoking habit, occupation, dietary habits, education, and income levels as presented in Table I.

Based on the statistical analysis result of the characteristics and demography of the research respondents (Table I), the community of Banjaroyo village mostly performed vigorous physical activity. There were 126 women (51.9%) and 78 men (32.1%) who did vigorous physical activity. There were 31 women (12.8%) did a low-moderate physical activity, whereas only eight men (3.3%) did a low-moderate physical activity. Based on the chi-square test, there is a significant relationship between physical activity and gender (p=0.034). The result is in line with previous researchers suggesting that women tend to do lighter physical activity than men (Sudikno et al., 2010; Sunu et al., 2017). Low physical activity leads to a greater chance of being obese (Diana et al., 2013; Wanner et al., 2016; Sudikno et al., 2010; Sunu et al., 2017; Sidik and Rampal, 2009; Nurzakiah et al., 2010).

There were 83 women (34.2%) and 25 men (10.3%) who were obese in Banjaroyo

village. Meanwhile, there were 74 women (30.5%) and 61 men (25.1%) who were not obese. Based on the chi-square test that has been conducted, there is a significant relationship between obesity and gender (p<0.001). The result is corroborated by previous researchers suggesting that the obesity proportion on women is greater than in men (Nurzakiah et al., 2010; Riebe, 20; Sudikno et al., 2010; Sidik and Rampal, 2009; Sunu et al., 2017; Fenty et al., 2016).

The respondents' dietary habits in Banjaroyo village are classified based on the consumption of vegetables and fruits, namely Less and Enough. There were 156 women (64.6%) and 85 men (35%) who had a habit of consuming vegetables and fruits. In average, the income level of the community of Banjaroyo village is in the middle level. It is proven by 116 women (47.7%) and 42 men (17.3%) who had low income (0-500,000 IDR). There were 41 women (16.9%) and 44 men (18.1%) who had high income (>500,000 IDR) (Sudikno et al., 2010). Based on the chi-square test that has been conducted, there is a significant relationship between income and gender (p<0.001).

Table II shows that there is an insignificant relationship between physical activity and body fat percentage (p=0.419; CI 95%=0.66-2.689). The result is in line with some researchers suggesting that there is an

insignificant relationship between physical activity and obesity (Dewi and Mahmudiono, 2012; Joh et al., 2013; Novitasary et al., 2013; Veghari et al., 2010; Tan and Yim, 2010). Meanwhile, the result is different from the research conducted by Rachmi and Allison (2017) which state that there is a significant relationship between less physical activity and obesity rate in Indonesia. The low level of physical activity is associated to have high risk in suffering obesity for men in Malaysia, but it does not apply to women (Chan et al., 2017).

Obesity is a complex problem caused by behavior, psychology, environment, and genetic factors (Chan et al., 2017). Obesity is not always associated with low physical activity. Energy that has been produced when doing physical activity shows that there is no difference for the subject who has a normal weight and is obese. Individual who is obese tends to be less physically active rather than the one who has normal weight (Westerterp, 2013). Obesity can happen because of the amount of energy in the form of food is greater than the amount of energy produced. The low energy production which is accompanied with excessive food consumption will increase the weight (Swift et al., 2014). The important consideration about the relationship between physical activity and obesity is the presence of hormones that can stimulate food entering the body. This will affect the balance of energy that is still not fully understood (Cook and Schoeller, 2011).

Others factors influencing obesity are gender, age, dietary habits, and smoking habits. Based on the table III, factor of gender and smoking habit have significant relationship to the obesity ( $p < 0.05$ ). This result is in accordance with the research conducted by Fenty et al. (2016), which states that in rural area of Yogyakarta, the prevalence of obesity among women is higher than men. Fat body can decrease when the physical activity is doing in high intensity. Meanwhile, women tend to compensate energy produced with increased food. Generally, women are not really losing body fat even though they do intensive exercise (Westerterp, 2013). In addition, smoking habit

also has a significant relationship to obesity ( $p < 0.05$ ). This is in line with the research conducted by Clair et al., (2011) who suggest that smoking habit at least one cigarette per day is positively associated with abdominal fat increase. Active smokers are more likely to have an unhealthy lifestyle such as less physical activity, high consumption of alcohol and less consumption of vegetables and fruits which easily cause fat accumulation in abdominal area.

## CONCLUSION

Based on the research that had been done, it can be concluded that there is no significant relationship between physical activities and the incidence of obesity among the community in Banjaroyo Village, Kalibawang, Kulon Progo, Special Region of Yogyakarta ( $p = 0.419$ ). Women have 3.12 times greater of suffering obesity than men. It is also applied for the respondents who do not smoke in which they have 2.95 times greater risk of suffering obesity than the respondents who do smoke.

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## THE EFFECT OF CANDESARTAN PRE-STROKE USE ON THE FUNCTIONAL OUTCOME OF POST ISCHEMIC STROKE PATIENTS IN BETHESDA HOSPITAL YOGYAKARTA

### PENGARUH RIWAYAT TERAPI CANDESARTAN PRASTROKE TERHADAP LUARAN FUNGSIONAL PASIEN STROKE ISKEMIK DI RUMAH SAKIT BETHESDA YOGYAKARTA

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#### ABSTRACT

*Stroke is a leading cause of death and disability, where the main risk factor is hypertension. Angiotensin Receptor Blocker (ARB) is the most common drug for stroke prevention in high-risk hypertensive patients. The purpose of this study was to see whether the candesartan pre-stroke use can improve the functional outcomes of post ischemic stroke patients. The data were obtained from 191 retrospective observational studies. Data were collected from Stroke Registry and medical record at Bethesda Hospital Yogyakarta in 2014-2016, then analyzed univariate, followed by bivariate analysis using chi-square test, independent t-test and fisher exact test for the variable which has actual count (F0), and logistic regression for multivariate analysis. One hundred and ninety one samples were systematically reviewed to evaluate the effect of candesartan pre-stroke use on functional outcomes of post ischemic stroke patients in Bethesda hospital Yogyakarta whose range of ages was mostly between 61 and 70 years (30.9%) and were mostly male patients (56.5%). Patients with good functional outcomes (<2) were 79.6% and poor functional outcomes (≥2) were 20.4%. The results of bivariate analysis showed that candesartan did not affect the improvement of clinical outcome (OR: 1.806, 95% CI: 0.591-5.519, p: 0.294), and also not better than other angiotensin receptor blocker (p=0.505, OR=1.472, 95% CI= 0.470-4.611). The multivariate analysis showed that sex (OR: 0.366, 95% CI: 0.156-0.858, p: 0.021), loss of consciousness (OR: 0.107, 95% CI: 0.021-0.549, p: 0.007), limb weakness (OR: 0.236, 95% CI: 0.067-0.834, p: 0.025), dyslipidemia comorbidity (OR: 2.750, 95% CI: 1.177-6.427, p: 0.019) and aphasia (OR: 0.342, 95% CI: 0.107-1.100, p: 0.072) affected the functional outcome. The candesartan pre-stroke use did not improve the functional outcome of post ischemic stroke patient.*

**Keywords:** *angiotensin receptor blocker, candesartan, functional outcome, ischemic stroke, neuron protection*

#### ABSTRAK

*Stroke merupakan masalah neurologis yang menyebabkan kecacatan serta kematian yang tinggi, dimana faktor utama penyebabnya adalah hipertensi. Angiotensin Receptor Blocker (ARB) adalah salah satu obat pilihan utama untuk prevensi stroke pada pasien hipertensi. Tujuan dilakukannya penelitian ini adalah untuk melihat apakah riwayat terapi candesartan prastroke dapat memperbaiki luaran fungsional pasien paska stroke iskemik. Penelitian ini dilaksanakan*

dengan menggunakan metode kohort retrospektif. Data diambil dari Stroke Registry dan rekam medis di Rumah Sakit Bethesda Yogyakarta pada tahun 2014-2016, kemudian dianalisis secara univariat, dilanjutkan analisis bivariat menggunakan uji chi-square test, uji-t independen dan uji fisher exact pada variabel yang memiliki actual count (F0), serta regresi logistik untuk analisis multivariat. Total jumlah sampel yang diambil pada penelitian adalah sebanyak 191 sampel dengan proporsi rentang usia terbanyak 61-70 tahun (30.9%) dan berjenis kelamin laki-laki (56.5%). Pasien dengan luaran fungsional baik (<2) adalah 79.6% dan luaran fungsional buruk (≥2) sebanyak 20.4%. Analisis bivariat menunjukkan bahwa candesartan tidak mempengaruhi perbaikan luaran fungsional pasien (OR:1.806, 95%CI:0.591-5.519, p:0.294) dan tidak lebih baik daripada obat ARB yang lain (p=0.505, OR=1.472, 95% CI= 0.470-4.611). Hasil multivariat menyatakan jenis kelamin (OR:0.366, 95%CI:0.156-0.858, p:0.021), penurunan kesadaran (OR:0.107, 95%CI:0.021-0.549, p:0.007), kelemahan anggota gerak (OR:0.236, 95%CI:0.067-0.834, p:0.025), komorbid dislipidemia (OR:2.750, 95%CI:1.177-6.427, p:0.019) dan afasia (OR:0.342, 95%CI:0.107-1.100, p:0.072) mempengaruhi luaran fungsional pasien. Riwayat terapi candesartan prastroke tidak memperbaiki luaran fungsional pasien setelah stroke.

**Kata kunci:** angiotensin receptor blocker, candesartan, luaran fungsional, stroke iskemik, proteksi neuron

## INTRODUCTION

Stroke is a neurological problem that causes disability and death and ranks as the first cause of deaths in Indonesia (CDC, 2016). Subarachnoid hemorrhage strokes are found on 1.4% of patients, intracerebral hemorrhage strokes are found on 18.5% of patients, and ischemic strokes are found on 42.9% of patients (Kusuima, 2009). Hypertension is one of the main risk factors that a person may experience a stroke, specifically in the Special Region of Yogyakarta, the number of hypertension increases from 8.3% in 2007 to 12.8% in 2013. Therefore, to control hypertension is needed to prevent the incidence of stroke (Yastroki, 2016; Riset Kesehatan Dasar, 2013). The effects of several anti-hypertension options are more than simply lowering down the blood pressure, such as ARB which protects the brain by its mechanism to lower the blood pressure, but also by the AT 2 receptor's improved performance which occurred due to the blockade of AT 1 receptor.

Angiotensin receptor blockers have been examined to have anti-inflammatory effects on the brain that mostly occur in the stroke patients, the two main processes that cause brain inflammation are peripheral infection

and direct cell injury as a consequence of metabolic changes in the brain parenchyma while the presence of ischemia, however, in each ARB drug option is believed to have different efficacy in reducing the brain inflammatory of the ischemic stroke patients. Candesartan is one of the ARB drug options that is still controversial in recovering the functional outcomes of the ischemic stroke patients so that the researchers are keen to assess whether candesartan is able to improve the functional outcomes of the post ischemic stroke patients (Chrysant and Chrysant, 2006; Tziomalos et al., 2014).

## METHODS

The research was carried out at the Bethesda Hospital, Yogyakarta, starting from February to April 2017, by using the retrospective cohort study as the method. Ischemic stroke patients were divided into two main groups namely the group of patients with the record of using pre-stroke candesartan and the group of patients with the record of using other anti-hypertension besides candesartan. The selection of the patients was conducted by using the purposive sampling technique in accordance with the existing criteria of inclusion and exclusion. The selected samples

were the patients who had been diagnosed using the cranial CT scan; who had the results of modified ranking scale (mRS) upon discharge; who had experienced the primer stroke onset and at the onset of less than 24 hours when hospitalized; and whose record of patients' data showed that they used angiotensin receptor blocker (ARB) type of anti-hypertension before the stroke onset, which was taken and then a sub-analysis was conducted to assess whether the candesartan was better than the other ARB drug choices. The patients who returned home at their own request, referral patients, and patients with incomplete medical record, were not taken as samples.

The research was conducted by using the instruments, such as patients' data forms and stroke registry with a minimum 53 samples per group. However, after the research was running, the comparison was altered into 1:4 so that the minimum number of the candesartan group was 21 patients and non-candesartan of anti-hypertension group was 85 patients (Sullivan and Soe, 2007). Then, the obtained samples were analyzed using univariate analysis in order to look at the samples' characteristics, followed by a bivariate analysis and then multivariate analysis to look at which variables that affected the patients' functional outcome significantly.

The data processing was conducted in the Clinical Epidemiology and Biostatistics Unit (CE&BU) Faculty of Medicine Gadjah Mada University by firstly taking the samples based on the criteria of inclusion i.e. all ischemic stroke patients diagnosed by using cranial CT scan and having the mRS results upon discharge so that the patients who were forced to discharge were excluded from the research. The selected patients had to have a candesartan therapy record and other pre-stroke hypertension and had experienced primer stroke with the onset of less than 24 hours. The referral patients from other hospitals, patients having incomplete medical data record, and those who were not recorded in the registry data had been excluded from the research.

## RESULTS AND DISCUSSION

There were 191 samples analyzed by using a descriptive, bivariate and multivariate analysis. Based on Table I, the data of patients with a good functional outcome ( $mRS < 2$ ) was obtained, as much as 79.6% (152 samples) and a poor functional outcome ( $mRS \geq 2$ ) with the amount of 20.4% (39 samples) or 56.5% of male and 43.5% of female. The analysis was followed up by using a bivariate analysis and then multivariate analysis aimed to analyze the significance of the variables toward ischemic stroke patients' functional outcome recovery. At this stage, the significance of the variables was seen from the value of  $p$ , if the value of  $p < 0.05$ , the variables were declared significant. On the contrary, if the value of  $p > 0.05$ , the variables were declared not significant.

The total number of the samples taken in this research was 191 samples and as presented in Table I, it was seen that the incidence of stroke commonly occurred at the age between 61 and 70 years (30.9%), in which 56.5% of the patients were male. The stroke onset events were mostly at the duration of 3-6 hours (31.9%) and 93.7% of the total samples did not experience loss of consciousness, 170 samples (89%) experienced aphasia and 144 samples (75.4%) experienced limb weakness. The patients having dyslipidemia comorbidities (52.9%) were more than those having comorbidities of atrial fibrillation (3.1%), while the total number of the patients experiencing complications was 83 samples (43.5%), the number of patients having complications on urinary tract infection and decubitus was 2 samples (1%) of the total samples on each category. The total number of patients who got neuroprotection therapy was 37.7% of the total samples and the patients with a record of using pre-stroke candesartan were to 15.7%, while the percentage of patients recorded to have used other anti-hypertension was 89.5% in which the sub-analysis was conducted to those two groups on the patients with a record of using only ARB drugs and then compared to the other ARBs to assess whether candesartan was a better choice than the other ARBs.

**Table I.** Basic Characteristics of the Research Subjects

The Characteristics of the Patients	n= 191	(%)
<b>Ages</b>		
< 40 years old	2	1.0
40-50 years old	32	16.8
51-60 years old	56	29.3
61-70 years old	59	30.9
>70 years old	42	22.0
<b>Gender</b>		
Female	83	43.5
Male	108	56.5
<b>Candesartan</b>		
Yes	30	15.7
No	161	84.3
<b>Anti-hypertension non- candesartan</b>		
Yes	171	89.5
No	20	10.5
<b>mRS Nominal</b>		
good mRS	152	79.6
bad mRS	39	20.4
<b>Onset</b>		
<3 hours	34	17.8
3-6 hours	61	31.9
6-12 hours	49	25.7
12-24 hours	47	24.6
>24 hours	0	0
<b>Loss of Consciousness</b>		
Yes	12	6.3
No	179	93.7
<b>Aphasia</b>		
Yes	21	11.0
No	170	89.0
<b>Limbs weakness</b>		
Yes	144	75.4
No	47	24.6
<b>Dyslipidemia Comorbidities</b>		
Yes	101	52.9
No	90	47.1
<b>Comorbidities of Atrial Fibrillation</b>		
Yes	6	3.1
No	185	96.9
<b>Complications</b>		
Yes	83	43.5
No	108	56.5
<b>Urinary Tract Infection Complications</b>		
Yes	2	1.0
No	189	99.0
<b>Decubitus Complications</b>		
Yes	2	1.0
No	189	99.0
<b>Neuroprotection</b>		
Yes	72	37.7
No	119	62.3
<b>Hospitalization Duration</b>		
>5 days	82	42.9
≤5 days	109	57.1

Based on Table II, some variables that were considered significant were the gender (p:0.028), loss of consciousness (p:0.000), aphasia (p:0.007), limb weakness (p:0.020), dyslipidemia comorbidities (p:0.002),

decubitus complication (p:0.005) by using fisher's exact test, the duration of hospitalization (p:0.023), meanwhile the record of candesartan therapy as a free variable in this study showed the results of



being not significant (p:0.294, OR: 1.806, 95%CI:0.591-5.519), as in other anti-hypertension types (p:0.961, OR: 0.971, 95%CI:0.305-3.089). Moreover, the sub-analysis was performed in the patients with a history of using only ARB therapy to assess whether the candesartan gave a better result of functional recovery compared to the other ARB medication choices and the results of the analysis in Table III showed that candesartan was not better than other ARB medication options (p:0.505, OR:1.472,95%CI:0.470-4.611).

Invalidity of a history on the use of ARB in patients' functional recovery was in line with the research conducted by Sandset et al. in 2011 involving 2029 patients from 146 centers in nine Northern European countries in a cohort prospective study. This study stated that the analysis of the functional outcome referred to the risk of a poor functional outcome on the given candesartan group (p: 0.048, OR:1.17,95% CI:1.00-1.38), the researchers even stated that 2% of the total patients experienced hypotension and renal failure, in which this value is bigger compared to the placebo group that is only 1% (Sanset et al., 2011).

The activation of central angiotensin II triggered a cerebral ischemia event. When the angiotensin II bound up with AT1 receptor, there would be a sympathetic effect triggered as the occurrence of vasoconstriction, the

proliferation of cells and the other AT1 receptor effects, so the use of candesartan would help restore the blood flow during the ischemia that the cerebrovascular auto-regulation process with a specific blockade on AT1 receptor became an important mechanism for the brain protection. However, the effectiveness of candesartan in recovering the functional for pre-therapy ischemic stroke patients was highly influenced by the state of blood-brain barrier on each patient. As described by Pelisch et al. (2010), candesartan had a limitation in penetrating the blood-brain barrier that was highly dependent on the dose, at the time allotment and liposoluble compounds, whereas the hypertension potentially caused cerebral ischemia and, on such circumstances, the angiotensin II was found that worsening the state due to unavoidable AT1 receptor activation (Pelisch et al., 2010). Candesartan itself had a low bioavailability, i.e. 40%, and it would be eliminated once it was injected to the blood systemic circulation mainly in the kidneys. The effective use of candesartan clinical dose ranged between 8 up to 32 mg per day, in which the monotherapeutic response levels of candesartan would rise up as the dose increased, but the absorption of candesartan never exceeded 60% so this would affect the efficacy of candesartan in pre-stroke therapy (Seltzer et al., 2004).

**Table II.** Predictive Factors of Functional Recovery of Ischemic Stroke Patients

Variables	mRS <2 (n=152)	mRS ≥2 (n=39)	OR	95% CI	p
<b>Candesartan Record</b>					
Yes	26 (13.6%)	4 (2.1%)	1.806	0.591-5.519	0.294
No	126 (66.0%)	35 (18.3%)			
<b>Non- Candesartan Anti-hypertension Record</b>					
Yes	136 (71.2%)	35 (18.3%)	0.971	0.305-3.089	0.961
No	16 (8.4%)	4 (2.1%)			
<b>Age (years old)</b>					
<40	2 (1.0%)	0 (0.0%)	Reff	Reff	0.764
40-50	27 (14.1%)	5 (2.6%)	1.56	0 – 23.56	

	51-60	45 (23.6%)	11 (5.8%)	1.28	0 – 16.27
	61-70	47 (24.6%)	12 (6.3%)	1.23	0 – 15.47
	>70	31 (16.2%)	11 (5.8%)	0.89	0
<b>Gender</b>					<b>0.028</b>
	Male	92 (48.2%)	16 (8.4%)	0.454	0.222-0.928
	Female	60 (31.4%)	23 (12.0%)		
<b>Onset</b>					<b>0.29</b>
	< 3 hours	26 (13.6%)	8 (4.2%)	Reff	Reff
	3-6 hours	46 (24.1%)	15 (7.9%)	0.94	0.31 – 2.79
	6-12 hours	38 (19.9%)	11 (5.8%)	1.06	0.33 – 3.37
	12-24 hours	42 (22.0%)	5 (2.6%)	2.58	0.67 – 10.37
<b>Decreased of Consciousness</b>					<b>&lt;0.001</b>
	Yes	4 (2.1%)	8 (4.2%)	0.105	0.030-0.370
	No	148 (77.5%)	31 (16.2%)		
<b>Aphasia</b>					<b>0.007</b>
	Yes	12 (6.3%)	9 (4.7%)	0.286	0.111-0.739
	No	140 (73.3%)	30 (15.7%)		
<b>Limbs Weakness</b>					<b>0.02</b>
	Yes	109 (57.1%)	35 (18.3%)	0.29	0.097-0.864
	No	43 (22.5%)	4 (2.1%)		
<b>Dyslipidemia Comorbidities</b>					<b>0.002</b>
	Yes	89 (46.6%)	12 (6.3%)	3.179	1.497-6.747
	No	63 (33.0%)	27 (14.1%)		
<b>Comorbidities Atrial Fibrillation</b>					<b>0.425</b>
	Yes	4 (2.1%)	2 (1.0%)	0.5	0.088-2.835
	No	148 (77.5%)	37 (19.4%)		
<b>Complications</b>					<b>0.985</b>
	Yes	66 (34.6%)	17 (8.9%)	0.993	0.489-2.019
	No	86 (45.0%)	22 (11.5%)		
<b>Urinary Tract Infection Complication</b>					<b>0.297</b>
	Yes	1 (0.5%)	1 (0.5%)	0.252	0.015-4.116
	No	151 (79.1%)	38 (19.9%)		
<b>Decubitus Complications</b>					<b>0.005</b>
	Yes	0 (0.0%)	2 (1.0%)	0.08	0 - 0.93
	No	152 (79.6%)	37 (19.4%)	Reff	Reff
<b>Neuroprotection</b>					<b>0.912</b>
	Yes	57 (29.8%)	15 (7.9%)	0.96	0.465-1.980
	No	95 (49.7%)	24 (12.6%)		
<b>Hospitalization Duration</b>					<b>0.023</b>
	>5 days	59 (30.9%)	23 (12.0%)	2.266	1.107-4.639
	≤5 days	93 (48.7%)	16 (8.4%)		

**Table III.** Sub Analysis of Candesartan VS ARB Non- Candesartan

Variables	mRS <2 (n=132)	mRS ≥2 (n=28)	OR	95% CI	p
Record of ARB Non- Candesartan	106 (66.3%)	24 (15.0%)	1.472	0.470-4.611	0.505
Record of Candesartan	26 (16.3%)	4 (2.5%)			

**Table IV.** Multivariate Analysis

Outcome	OR	95% CI		P
		Lower	Upper	
Gender	0.366	0.156	0.858	0.021
Decrease of Consciousness	0.107	0.021	0.549	0.007
Aphasia	0.342	0.107	1.100	0.072
Limbs Weakness	0.236	0.067	0.834	0.025
Dyslipdemia	2.750	1.177	6.427	0.019
Duration of hospitalization	1.305	0.554	3.074	0.543
Decubitus Comorbidities	0.000	0.000	-	0.999

Sandset et al. (2015) stated that candesartan recovered the patients' functional outcome, but its efficacy also depended on the type of sub-types of ischemic stroke experienced by the patients, in which a better functional outcome tended to occur on the patients with lesions on the myocardial infarction on the total anterior circulation infarction (TACI) sub type or on the partial anterior circulation infarction (PACI) sub type rather than on lacunar infarction (LACI) (p:0.02). Thus, the pre-stroke hypertension patients who started experiencing ischemic on the anterior circulation on either partial or total would be greatly helped with the use of candesartan because if there was a bigger infarction on TACI and PACI, the patients became vulnerable to the development of cerebral edema or the possibility of the hemorrhage occurrence. This type of infraction was more commonly caused by atherothrombotic or cardioembolic that were supported by the prevalence of atrial fibrillation which was higher for the patients with this infarction sub type. Thus, it made sense if reducing the blood pressure on this sub type would avoid the risk, even though the candesartan in doing brain protection was not as simple as reducing the patients' blood pressure. As explained in the previous chapter,

the candesartan just blocked the AT1 receptor so it meant that AT2 receptor that had an opposite working mechanism would greatly help protect the brains. Most patients who experienced LACI were chronic hypertension sufferers who eventually showed an auto-regulation ability in regulating brain blood regulation. It was believed that reducing the blood pressure would cause blood perfusion deficits broadly on the brain because the blood flow became inadequate in doing brain tissue perfusion (Sandset et al., 2015). On the other hand, these things were the reasons why the patients with the candesartan record did not show functional outcome improvement. In this research, the dose of therapy and the obedience of each patient could not be researched, as supported by the candesartan's limitation in penetrating the blood-brain barrier and the wide ischemic that occurred when the patient experienced hypertension.

The results of this study indicated that the male patients had lower functional outcome probability than female patients. This contrasted to the Ng et al. (2013) who stated that female patients had worse post-stroke physical ability than male patients who were able to do physical activity independently (HR: 0.51, 95%CI:0.32-0.79). The researcher stated that the pre-stroke physical function and

the symptoms of depression were important factors in the different results of the both gender recovery (Ng et al., 2013). Some researches indicated that female experienced more severe stroke compared to male even though other studies indicated that there was no significant difference in both genders. The International Stroke Trial found that the incidence of death on female was high in 6 months after the stroke. This was corroborated by Gargano and Reeves (2007) who agreed that female had lower functional outcome and a poor quality of life compared to male after a devastating stroke. Meanwhile, The Women's Health Organization MONICA Project found that within 28 days of observation on ischemic stroke patients, the mortality rate of female was on par or higher than male. However, it was in contrast to the latest research from Framingham Heart Study which found that there was no significant difference on mortality of both genders. The fundamental difference between male and female such as age, comorbidity, severity, and pre-stroke disability commonly caused deaths on female, but even when these factors had been controlled, female still had worse functional outcome (Gargano and Reeves, 2007; Persky et al., 2010; Min et al., 2005).

Patients who experienced aphasia independently had probability of having good functional outcome 0.342 times compared to those who did not experience it (OR:0.342), however, Maas et al. (2012) stated that the evaluation results of ischemic stroke patients who suffered aphasia after 6 months would have a recovery of 86% and 74% (Maas et al., 2012). Based on the results of the multivariate analysis (Table IV), it was clearly presented that the patients entering the hospital with decreased consciousness had the probability of having worse functional outcome than those who did not experience it (OR:0.107), Tsao et al. (2005) in their study stated that the patients with a good level of consciousness during the attack was related with a good functional outcome of the patients (Tsao et al., 2005). The patients who enter the hospital with decreased consciousness described the stroke severity they experienced and the wider

lesions, the more severe brain hypoxia occurred so that this would influence the extent of the penumbra that could be saved in order to get the patients a better functional outcome.

Patients with dyslipidemia had the 2.75 times probability of having a good functional outcome compared to those who did not experience it. Out of the patients with dyslipidemia comorbidities, 89 patients had a good functional outcome while 12 patients had a bad functional outcome. In the patients without comorbidities, there were 63 patients with a good functional outcome and 27 patients with a bad functional outcome. A study on meta-analysis in 2007 stated that the total number of cholesterol did not take part significantly on the mortality of the ischemic stroke patients aged 40-59 years. However, it was strongly influenced by the patients' blood pressure. For the patients with blood pressure more than 145 mmHg, their cholesterol did not strongly affect the patients' mortality. It was estimated to be due to vascular collaterals on the brains, but a meta-analysis stated that decreasing the cholesterol levels might reduce the risk of stroke (Lewington et al., 2008). The multivariate analysis showed that the limb weakness was strongly affected by bad functional outcome (OR:0.236). Gray et al. (2012) stated that after a stroke, the patients would spend more than 50% of their time lying down. This inactivity caused sarcopenia to occur. Sarcopenia is the state in which the patients lose their muscle mass causing them to gain more fat mass in the body. Within 2 weeks of inactivity, it had already caused the loss of muscle mass and its strength. Thus, the damage caused by stroke and immobility would simultaneously cause the loss of muscle mass and impact on the patients' independence in doing physical activities (Gray et al., 2012).

Long hospitalization did not improve the patients' functional outcome. This finding was contradicted by Bindawas et al. (2016) who stated that patients who were hospitalized for a long time were closely related to comorbid disease that accompanied it. Therefore, it was common that the patients'

functional outcome became worse, whereas the patients with a short and medium time of hospitalization had better functional outcome. In this state, the patients were considered as having an efficient medication so that they had shorter time of hospitalization. However, the decision regarding to the length of hospitalization extremely varied to adjust to the type of insurance service applied by the corresponding health services so that it was reasonable that the duration of hospitalization could not be a specific benchmark in determining the patients' functional outcome when they were out of the hospital. The length of hospitalization could usually be prolonged under a certain condition of the patients. Wang et al. (2014) concluded that the patients who underwent a decubitus rehabilitation would experience motor skill declining and likely to enter into the community (Wang et al., 2014; Bindawas et al., 2016).

## CONCLUSION

Based on the analysis and the discussion above, it is concluded that the use of candesartan therapy before the onset of stroke does not affect patients' clinical improvement after a stroke.

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## A SURVEY OF ANTIBIOTICS PURCHASED WITHOUT PRESCRIPTION AMONG NON-HEALTH SCIENCE STUDENTS IN JEMBER, INDONESIA

## SURVEI PEMBELIAN ANTIBIOTIKA TANPA RESEP DI KALANGAN MAHASISWA NON-KESEHATAN DI JEMBER, INDONESIA

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### ABSTRACT

Previous research showed that self-medication practice with antibiotics is widespread in developing countries and tertiary students are among these consumers. This study aimed to estimate the prevalence of the use of antibiotics without prescription among non-health science students in Jember and to identify the sources of non-prescription antibiotics. This cross-sectional survey was conducted between April and June 2016 in all non-health science faculties of Universitas Jember. A questionnaire containing four main short questions about antibiotics use and questions related to socio-demographic information was distributed to students who gave consent. A number of 130 (58.5%) respondents admitted to ever acquire antibiotics without the presence of a physician prescription. Self-medication with antibiotics tended to be done by students from rural areas ( $p=0.04$ ) and without health insurance ( $p=0.04$ ). The sources of non-prescription antibiotics vary, but pharmacy was the most frequently cited to be the source ( $n=82$ ; 46.3%). This study showed that the use of antibiotics without prescription among non-health science students and the sale of antibiotics without prescription among pharmacies in Jember are prevalent. Real responses from the government, relevant health professional associations, and education institutions to intervene these problems are significantly needed.

**Keywords:** antibiotics, Jember, non-health science students, self-medication

### ABSTRAK

Beberapa penelitian menunjukkan bahwa praktik swamedikasi dengan antibiotik marak di kalangan masyarakat negara berkembang dan mahasiswa termasuk juga sebagai konsumen antibiotika dengan cara ini. Penelitian sederhana ini bertujuan untuk mengetahui prevalensi penggunaan antibiotika tanpa resep di antara mahasiswa non kesehatan di Jember dan untuk mengetahui sumber perolehan antibiotika tanpa resep. Survei potong lintang ini dilaksanakan antara April hingga Juni 2016 di semua fakultas non-kesehatan Universitas Jember. Kuesioner yang berisi empat pertanyaan utama tentang penggunaan antibiotika dan pertanyaan terkait informasi sosio-demografi dibagikan kepada mahasiswa yang memberikan consent. Sebanyak 130 (58,5%) responden mengaku pernah mendapatkan antibiotika tanpa resep dokter. Praktek swamedikasi dengan antibiotika cenderung dilakukan oleh mahasiswa yang berasal dari daerah rural ( $p=0,04$ ) dan yang tidak memiliki asuransi ( $p=0,04$ ). Responden mendapatkan antibiotik tanpa resep dari berbagai sumber dan apotek menjadi tempat paling sering disebutkan untuk membeli antibiotika tanpa resep ( $n=82$ ; 46,3%). Penelitian ini menunjukkan penggunaan antibiotika tanpa resep masih tinggi di kalangan mahasiswa non kesehatan dan penjualan antibiotika tanpa resep di apotek di Jember cenderung tinggi. Peran nyata pemerintah, organisasi

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**Kata kunci:** *antibiotika, Jember, mahasiswa non kesehatan, swamedikasi*

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## INTRODUCTION

The self-medication behavior, which is a part of self-care, is commonly practiced by the people of all ages (Bertoldi et al., 2014; Donkor et al., 2012; Du and Knopf, 2009; Hassali et al., 2011; Jerez-Roig et al., 2014) living in developed countries (Aoyama et al., 2012; Guinovart et al., 2015) and in developing countries (Bertoldi et al., 2014; Eticha and Mesfin, 2014), in the city (Selvaraj et al., 2014) and villages (Skliros et al., 2010) with "modern" medicine (Mehuys et al., 2014) and traditional medicine (Clement, 2009). Health complaints that are treated by self-medication vary from mild ones, such as headaches and fever (Eticha and Mesfin, 2014), to severe, such as depression (Khanra and Sen, 2016).

Self-medication involves the process of self-diagnosis that requires experience and sufficient knowledge. Therefore, one of the risks that can arise is misdiagnosis (Ruiz, 2010). Other problems such as medicine interactions may also happen. Self-medication action is said to be responsible if the medicine taken are the ones which do not need prescription from a doctor. Those medicine include the free or over-the-counter (OTC) medicine, Pharmacy-Prescribed-Medicine (OWA), and traditional medicine. However, considerable evidence from previous studies has shown that prescription only medicine is also used in self-medication, one of which is antibiotics (Donkor et al., 2012; Guinovart et al., 2015; Skliros et al., 2010). The purchase of antibiotics without prescription may exacerbate the control of antimicrobial resistance rates. This is a serious global threat. Although the contribution of the pattern of antibiotics purchased without prescription to the antimicrobial resistance is not very clear, but this phenomenon is thought to play an important role (Morgan et al., 2011).

Antimicrobial resistance is one of the most important health issues known to those with a background in health, including health students. This knowledge is thought to be able to make health students more alert to self-medication using antibiotics without a prescription. However, some previous studies have shown inconsistent results (Donkor et al., 2012; Olayemi et al., 2010). A study in Ghana shows that the prevalence of self-medication using antibiotics in health students is relatively lower than in non-health students (Donkor et al., 2012), whereas a study in Nigeria shows otherwise (Olayemi, 2010). A study in Karachi shows a high prevalence of self-medication using antibiotics among students (Shah et al., 2014). Some studies of self-medication conducted on health students, particularly on the pharmacy students, have shown quite interesting results, because although the students have good knowledge, but in practice they have not demonstrated a wise use of antibiotics (Dhingra et al., 2015; Fejza et al., 2016). In the study, researchers suggested to conduct intervention or training activities to increase the use of antibiotics wisely among students. Based on the results of the study, a self-medication study on the use of antibiotics among non-health students becomes an interesting topic. Since the practice of irresponsible use of antibiotics occur in health students, it can be assumed that the non-health students also have a great risk of doing the same practice. This study was conducted to find out the prevalence of self-medication using antibiotics in non-health students and to figure out the places where antibiotics can be purchased without a prescription.

## METHODS

This study was conducted using a paper-based interview and face-to-face interview in



April-June 2016. The respondents were selected using a convenient sampling technique. Respondents were taken from ten non-health faculties of Universitas Jember, namely Faculty of Law, Faculty of Social and Political Sciences, Faculty of Agriculture, Faculty of Economics, Faculty of Teachers Training and Education, Faculty of Letters, Faculty of Agricultural Technology, Faculty of Mathematics and Natural Sciences, Faculty of Engineering, and Faculty of Information System. The inclusion criteria included undergraduate (S-1) students who were active from batch 2012-2015 and who were willing to sign the informed consent sheet. Respondents were excluded if they had a history of chronic disease requiring regular therapy or if they did not answer the questionnaire completely. Research permit was obtained from Vice Rector I of Universitas Jember (No. 4937/UN25/LT/2016). The identity of each respondent was kept confidential and the data were only accessible to the researchers.

The questionnaire consisted of two parts. Part A covered the demographic data of the respondents including gender, residence, pocket money, place of origin, and type of insurance. Part B covered four questions related to self-medication using antibiotics. The first question was about whether the respondents consumed or used antibiotics. Some examples of antibiotics, namely "amoxicillin", "super tetra" and "cefadroxil" were included in this question as what was conducted in Pakistan (Gillani et al., 2017). Three choices of answers which were "Yes", "No", and "Do Not Know" were provided to answer the first question. When answering "Yes", respondents were asked to answer three other questions. The second question was about the type of antibiotics ever used. Respondents may mention more than one type of antibiotics to answer this question. The third question was about whether the antibiotics were obtained without a prescription. Two choices of answers, which were "Yes" and "No", were provided to

answer the third question. If they answered "Yes", the respondents did not need to continue answering the next question. Nevertheless, if they answered "No", the respondents were required to answer the last question. The last question was about the place where they purchased the antibiotics. The choices of answers for this fourth question included "Pharmacy", "Grocery Store", "Friend/Family", "Drug Store", "Doctor Practice", "Midwife Practice", "Health Care", "Supermarket", and "*Mantri*". Pre-testing of the questionnaire was conducted by recruiting 30 respondents before the actual survey to determine the face validity and the content validity of the questionnaire. Reliability test was also conducted at the pre-testing stage by using the test-retest method with a time gap of 14 days.

Antibiotics without prescription are defined as antibiotics obtained from a variety of sources without the use of legitimate prescriptions from authorized health personnel and without going through legal distribution channels. Self-dispensing medicine by self-employed practices of health workers such as doctors, midwives, and *mantri* are included within these limits. Supermarkets, left over medicine at home, and health care without prescribing doctor services are included as the sources to obtain antibiotics without a prescription. The practice of using antibiotics without a doctor's prescription is also referred to as self-medication using antibiotics. The prevalence of consuming antibiotics without prescriptions or antibiotics self-medication was calculated from the number of respondents who claimed to purchase antibiotics without a prescription divided by the total of respondents who had used antibiotics. The data was recorded into Stata version 13 (Stata Corp., College Station, Texas, USA). The data were analyzed statistically using Chi-Square or Fisher's exact test with a 0.05 significance level to determine the correlation between socio-demographic factors as well as antibiotics use experience and prescription use to get the antibiotics.

**Table I.** The Respondents' Socio-demographic Characteristics

Characteristics	Ever consumed antibiotics? (N=280)			Purchasing antibiotics with prescription? (N=222)		
	Yes N=222 (%)	No N=58 (%)	p Value	Yes N=92 (%)	No N=130 (%)	p Value
Sex			0.82			0.81
Male	92 (79)	25 (21)		39 (42)	53 (58)	
Female	130 (80)	33 (20)		53 (41)	77 (59)	
Semester			0.12			0.90
2	66 (78)	19 (22)		26 (39)	40 (61)	
4	62 (85)	11 (15)		26 (42)	36 (58)	
6	48 (71)	20 (29)		22 (46)	26 (54)	
8	46 (85)	8 (15)		18 (39)	28 (61)	
Age			0.25			0.67
<20	55 (80)	14 (20)		20 (36)	35 (64)	
20-21	129 (77)	39 (23)		56 (43)	73 (57)	
>21	38 (88)	5 (12)		16 (42)	22 (58)	
Residence			0.01			0.43
Boarding house	156 (76)	50 (24)		62 (40)	94 (60)	
Home	66 (89)	8 (11)		30 (45)	36 (55)	
Place of Origin			0.25			0.04
Urban	95 (83)	20 (17)		47 (49)	48 (51)	
Rural	127 (77)	38 (23)		45 (35)	82 (65)	
Insurance participants			0.01			0.04
Yes	86 (88)	12 (12)		43 (50)	43 (50)	
No	136 (75)	46 (25)		49 (36)	87 (64)	
Monthly allowance (in thousands of Rupiah)			0.25			0.38
<500	86 (83)	17 (17)		37 (43)	49 (57)	
500-1.000	101 (74)	35 (26)		40 (40)	61 (60)	
1.000-1.500	24 (86)	4 (14)		8 (33)	16 (67)	
>1.500	11 (85)	2 (15)		7 (64)	4 (36)	

**RESULTS AND DISCUSSION**

From the total of 324 non-health students participating in this study, 222 students (68.5%) admit to having used antibiotics (Figure 1A). Among those who have used antibiotics, the type of antibiotics used is relatively limited. Nearly 90% of the students (n=199) state that they had ever used only one type of antibiotics and none of the students

consume more than two types of antibiotics. From the total of 245 mentions, amoxicillin (173 times, 70.6%) is the most commonly mentioned antibiotics (Figure 1B). These results are consistent with the results of the study conducted among the students in Accra, Ghana (Donkor et al., 2012), Karachi, Pakistan (Shah et al., 2014), and Ahwaz, Iran (Sarahroodi et al., 2010) which also put

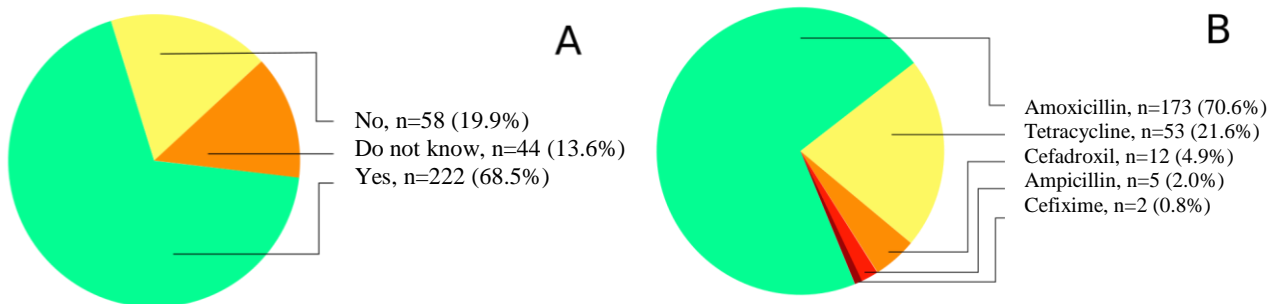
amoxicillin on the first rank. The respondents of this study only mentioned five types of antibiotics, all of which are limited in the beta-lactam and tetracycline groups. Studies conducted by Shah et al. (2014) and Gillani et al. (2017) each mentions seven types of antibiotics used in self-medication by students. Sarahroodi et al. (2010) and Donkor et al. (2012) reported even more types of antibiotics, which are eight and 13 antibiotics types.

The prevalence of consuming antibiotics without prescription in non-health student respondents was 58.6% (Table I). This study is in line with the results of other studies showing that the prevalence of self-medication using antibiotics is still relatively high. One of the examples is from the study conducted on the students in Punjab, Pakistan which is 45% (Gillani et al., 2017) and in Accra, Ghana which is 70% (Donkor et al., 2012). Self-medication practices using antibiotics without a doctor's prescription are not significantly different based on the sex ( $p=0.81$ ), batch ( $p=0.90$ ), age ( $p=0.67$ ), residence ( $p=0.43$ ) and the amount of allowance ( $p=0.38$ ). However, self-medication practices using antibiotics are associated with the students' place of origin ( $p=0.04$ ) and insurance membership ( $p=0.04$ ) (Table I). Students who have an insurance are

less likely to practice self-medication using antibiotics compared to those who do not have an insurance (50% vs 64%). This study is in line with the study conducted in Yogyakarta, Indonesia which states that the respondents who do not have an insurance are 1.5 times more likely to practice self-medication (Widayati et al., 2011). Participation in health insurance may decrease self-medication practices (Pagán et al., 2006), this is possible because insurance participants feel the need to take advantage of facilities covered by insurance and insurance participants feel entitled to better health services.

**Table II.** Sources to Purchase Antibiotics Without Prescription

Source of antibiotics	Amount of mention (%)
Pharmacy	82 (46.3)
Grocery store	23 (12.9)
Friend/family	22 (12.4)
Drug store	18 (10.1)
Doctor practice	9 (5.0)
Midwife practice	9 (5.0)
Health care	6 (3.3)
Supermarket	5 (2.8)
Mantri	3 (1.6)
<b>Total</b>	<b>177 (100)</b>



**Figure 1.** Distribution of the respondents' answers for the question "Have you ever consumed or use antibiotics?" (A) and distribution of antibiotics types among non-health students who ever consumed antibiotics whether for self-medication or not (B). Every respondent may answer more than one type of antibiotics.

In general, the use of antibiotics without a prescription is a convenient practice in many parts of the world and with a very diverse prevalence (Morgan et al., 2011, Ocan et al., 2015). Morgan et al. (2011) estimates the use of antibiotics without prescription in Asia at around 4-75%. Ocan et al. (2015) estimates the same rate in the countries with low and middle income at the range of 38.8% (95% CI: 29.5-48.1). Although it is a common thing to do, such practices are not in accordance with the applicable law. In Indonesia, antibiotics are classified as strong medicine that can only be obtained through prescription, except for some types of antibiotics for certain routes and in certain amounts which are included in the Pharmacy-Prescribed-Medicine (OWA) list (Health Minister of the Republic of Indonesia, 1986; Minister of Health of the Republic of Indonesia, 1993; President of the Republic of Indonesia, 2009; Directorate General of Pharmaceutical Services and Medical Devices, 1949). Prescription access is very important to suppress the rate of antibiotics resistance, although there is currently a debate about the need for non-prescription access for certain therapies, such as urinary tract infections (Llor, 2015).

Related to the source of purchasing antibiotics without prescription, out of 177 mentions (Table II), pharmacies (n=82; 46.3%) are the most frequently mentioned source, followed by grocery stores (n=23; 13.0%) and friends/family (n=22; 12.4%). No respondents mentioned any additional sources other than the ten kinds of outlets provided in the questionnaire. If compared to results of the previous studies, sources other than pharmacies are usually ranked in varying degrees or proportions. However, pharmacies tend to rank highest as reported in three systematic reviews (Morgan et al., 2011; Shaghghi, 2014; Ocan et al., 2015). These results indicate that pharmacists in the community have an important role and a great opportunity to control the purchase of antibiotics without a prescription. It is important to note that in Indonesia, one of the most common sources of antibiotics without prescription is the roadside stalls, as reported

in the study conducted in Yogyakarta (Widayati et al., 2011) and in Surabaya (Hadi et al., 2010).

There are some limitations in this study that include the sampling process which uses the convenient sampling technique and not using the random sampling technique. However, to minimize the limitations, the respondents were taken in a considerable amount and the number of respondents was proportioned in accordance to the total number of students in each non-health study programs.

## CONCLUSION

The results of this study indicate that the use of antibiotics without prescription is still relatively high among non-health students and sales of antibiotics without prescription at pharmacies in Jember tend to be high. Therefore, the real actions from the government, relevant professional organizations, and educational institutions to conduct interventions are needed. Further research needs to be conducted to determine the factors that are associated with the high sales of antibiotics without prescription at the pharmacies.

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