Effectiveness of Ethanolic Extract Ketepeng cina Leaves (*Senna alata* L.) As Antidiabetic Activity Test In Male Wistar Rats Induced by Alloxan

To cite this article: Herlina *et al* 2019 *J. Phys.: Conf. Ser.* **1282** 012085

IOP Conf. Series: Journal of Physics: Conf. Series **1282** (2019) 012085 doi:10.1088/1742-6596/1282/1/012085

Effectiveness of Ethanolic Extract Ketepeng cina Leaves (*Senna alata* L.) As Antidiabetic Activity Test In Male Wistar Rats Induced by Alloxan

Herlina[1*], Annisa Amriani[1], Indah Solihah[1], Putri Damayanti[1], Sri Wahyuningsih Rais[2]

[1] Departement of Pharmacy, Faculty of Matemathics and Natural Sciences, Sriwijaya University
[2] Departement of Medical Dentist, Faculty of Medical, Sriwijaya University

*rinaafdil@gmail.com

Abstract. Antidiabetic activity test of ethanolic extract of ketepeng cina leaves (*Senna alata* L.*)* to rats induced by alloxan has been done. Male wistar albino rats weree used as animal models which divided into 6 groups, normal group (aquadest), negative control group (Sodium CMC 0,5%), positive control group (insulin by 1IU/kgBW), and 3 test group (ethanolic extract of ketepeng cina leaves 200, 400, dan 800 mg/kgBB). Rats blood glucose level after induced intraperitoneally by alloxan 130 mg/kgBB can be stated as diabetic when >200 mg/dL. Antidiabetic-activity was tested by measuring on blood sugar levels by using the DTN-410-K fotometer, on day 0, 5, 10, and 15. The result of AUC_{0-15} and percentage of decreasing blood sugar levels for positive control group are 2557,35 and 51,20%, and 3 treatment groups (200 mg/kgBW, 400 mg/kgBW, and 400 mg/kgBW) 2745,6 and 39,94%; 2618,55 and 47,54%; 2448,425 and 57,72%. This point indicated that the ethanolic extract of ketepeng cina leaves has an antidiabetic activity and there is no significant difference compared with insulin ($p<0,05$). 800 mg/kgBW dose is the effectivity dose from other treatment doses, because it had the lowest AUC_{0-15} and the highest perscentage of decreasing blood sugar levels. According to the relation between percentage of blood glucose decrease level with dose, value of ED_{50} of ethanolic extract of ketepeng cina leaves is 522.413 mg/kgBW.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease disorder caused by the pancreas not producing enough insulin or the body can not use effectively produced insulin. As a result there is an increase in blood glucose concentration (hyperglycemia). Type 1 diabetes is caused by a disturbance of insulin production due to the destruction of the Langerhans island β cells caused by an autoimmune reaction [1].

The mechanism of flavonoids as antidiabetes is thought to play a significant role in increasing the activity of antioxidant enzymes that can reduce oxidative stress and reduce reactive oxygen species (ROS) so as to have protective effect on pancreatic β cells and increase insulin sensitivity [2]. Therefore, in vivo test decreased aloksan-induced blood glucose in rats using 70% ethanol extract. Method of diabetes induction in animals ui using alloxan. This diabetogenic substance is selectively destructive to pancreatic β cells. Next will be measured blood glucose level by the enzymatic method of godpap (glucose oxidase phenol 4-aminophenazone). This study is expected to provide information

on the value of AUC (Area Under Curve) and percent decrease in blood glucose levels as an important parameter of antidiabetes so that it can be determined the percentage decrease in blood glucose levels.

2. Experimental Section

2.1. Materials and Chemicals

The equipment used in this study includes a set of laboratory glassware, DTN-410-K spectrophotometer, EDTA tube, 1000 µL, 100 µL and 10 µL pipettes, hematocrit pipettes, rotary evaporators and centrifuges, maseration tools, blenders and chamber.

The materials used in this research are Chinese ketepeng leaf (Senna alata L.), white male rats Wistar strain, 70% ethanol, injection syringe, per-oral or sonde syringe, GOD-PAP kit, alloxan, Human insulin, glucose standard, Na CMC 0.5%, distilled water, NaCl 0.9%.

2.2. Test Animals Preparation

The test animals used were male Wistar (*Rattus novergicus*) white rats weighing between 200-250 g and age between 2-3 months. The animals were allowed 1 week to acclimatize before the commencement of the experiment. The animals were kept in room temperature and given enough food and drink.

2.3. Production of Ketepeng cina Leaf Simplicia

Leaves of ketepeng cina weighed 2 kg were obtained from the Indralaya campus of Sriwijaya University, South Sumatra. The samples are weighed, sorted and washed to separate the dirt attached. The leaves drying process is done by air drying under the sun and covered with a black cloth to avoid direct sun exposure. After dryed, the sorting back process is done to obtain simplicia with good quality. After that, the dried leaves are pulverized using a blender and weighed as a net weight.

2.4. Preparation of Ketepeng cina Leaf Extract by Multistage Maceration

The extraction process is done by multilevel maceration method using solvent type that is 70% ethanol. The first meteration was performed using 1 kg of simplicia powder macerated in a sun-shielded glass container using a 70% ethanol solvent of 4 L for 2 x 24 hours. Remaseration is done every 1 x 24 hours maserat obtained then filtered with filter paper. The maserate of the solvent is concentrated by evaporating the solvent using a rotary evaporator at 70°C. calculated value percent yield of leaf extract of ketepeng china. Percent extract yield is calculated as follows:

$$\%Yield = \frac{weig\,ht\ of\ extract\ \ obtained}{weig\,ht\ of\ extracted\ \ simplicia\ \ powder} \times 100\% \quad (1)$$

2.5. Phytochemical Test of Ketepeng cina Leaf Simplicia

2.5.1. Flavonoids Identification. The phytochemical screening test of flavonoid test was carried out as much as 0.1 g of extract samples inserted into the test tube, added 5 mL of ethanol, and heated for 5 minutes. The extractions were then filtered and the filtrate was added a few drops of concentrated HCl. Thereafter incorporated approximately 0.2 mg of magnesium powder (Mg metal). If the red color appears then it shows the presence of flavonoid group compounds [3].

2.5.2. Phenolic and Tannins Identification. Phenolic and tannin tests were carried out as much as 0.1 g of ethanol extract sample dissolved in 10 mL aquades, heated 5 minutes and filtered. The added filtrate is added 4 - 5 drops of FeCl3. The presence of phenol and tannin is indicated by the formation of dark blue or greenish color.[4]

2.5.3. Alkaloids Identification. An alkaloid test of 0.1 g of ethanol extract sample was dissolved with 9 mL of distilled water and 1 mL of HCl 2 N, then heated over a water bath for 2 minutes, then cooled. Further filtered and filtrate was used as the experimental solution to be used in the following test: A total of 1 mL of filtrate was transferred to a porcelain plate to add 2 drops of Wagner reagent. Positive

results indicate the presence of brown deposits. A total of 1 mL of filtrate is transferred to a porcelain plate add 2 drops of Mayer reagent. Positive results indicate the presence of white deposits. A total of 1 mL of filtrate is transferred to a porcelain plate add 2 drops of Dragendorf reagent. Positive results indicate the presence of orange sediment.

2.5.4. Saponins Identification. Saponin test was performed as much as 0.1 g ethanol extract sample added 10 mL hot water. After that it is cooled and shake firmly. Arising froth or foam as high as 1 cm or more is stable and in addition 1 drop of 2N HCl foam does not disappear then positive saponin group [5]

2.5.5. Identification of Steroids andTriterpenoids. A total 0.1 g of ethanol extract sample were dissolved in chloroform. The result of the filter is piped 2 - 3 drops and left to dry on the drop plate. After dry add Lieberman-Burchad reagent (2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid). The formation of red, pink, or violet means positive terpenoid, whereas if it is green or blue it means positive for steroid [4].

2.6. Test Design inRats

Test animals, 24 male white rats were selected randomly, and then divided into 6 groups and 12 rats in 3 groups for testing of active extracts. Calculations based on Federer's (1991) formula are calculated by the equation 2[8]:

$$(n–1)(t–1)\geq 15 \quad (2)$$

Description: n = number of sample repetitions, t = number of treatment groups.

Based on calculations using the formula in Equation 5, the test animals used in this study were 4 rats per group. The test animals needed in this study were 36 male Wistar white rats, with details in Table 1.

Table 1. Test Animal Treatment Groups

Group	Treatment
Normal	Aquadest p.o
Negative Control	Alloxan 130 mg/kg BW ip + Sodium CMC 1% b/v p.o
Positive Control	Alloxan 130 mg/kg BW ip + Insulin 1 IU/kg BW
Group I	Alloxan 130 mg/kg BW ip + Ethanol extract 200 mg/kg BW p.o
Group II	Alloxan 130 mg/kg BW ip + Ethanol extract 400 mg/kg BW p.o
Group III	Alloxan 130 mg/kg BW ip + Ethanol extract 800 mg/kg BW p.o

2.7. Induction of Alloxan in Rats

All groups except the normal group were induced with alloxan doses of 130 mg / kgBW in intraperitoneal physiologic NaCl solution. Measurement of blood glucose levels three days after induction using a gluco-DR strip by taking a small amount of blood through a mouse's tail. If BGL <200 mg / dL then reunion of alloxane 130mg / kgBW until BGL constant> 200 mg / dL.

2.8. Antidiabetic Activity Test

Mice with blood glucose levels greater than 200 mg / dL were used to continue in antidiabetic testing. The division of animal group test as in Table 1 of each group was given treatment in each dosage of test dosage once every 1 day for 15 days. Oral test preparation was administered by the weight of the test.

2.9 . Measurement of Blood Glucose Level Method of GOD-PAP

Sampling of the rat direction through the retroorbital plexus of the eye vein by using a hemocyte pipette of 1 mL. blood is accommodated in an EDTA tube through the tube wall to prevent lysis. Blood is concentrated twice 2500 rpm for 10 minutes to get blood serum. Addition of GOD-PAP

reagent at incubation time at 25^{o}C for 15 minutes. Procedure examination of blood samples as seen in Table 2.

Table 2. Measurement procedures BGL GOD-PAP

Solution	Sample (mL)	Raw (mL)	Blanko (mL)
Blood Serum	10 (μL)	-	-
Standar Glucose	-	10 (μL)	-
Reactor	1,0	1,0	1,0

2.10. Data Analysis

Data of decreased blood glucose level was analyzed by descriptive normality test (Shapiro-wilk) to know the distribution of data. If the data proved to be normally distributed, then a parametric statistical analysis with one way ANOVA with 95% confidence level was performed. If the data obtained proved there are significant differences, then followed the LSD post hoc test. The program used for data processing is software SPSS® (for windows) version 23.

3. Result and Discussion

3.1. Extraction and phytochemical screening result

The result of plant determination used as research object is Senna alata L. from family of Fabaceae. The maceration method is chosen to separate the compound from the ketepeng cina leaf of according to its polarity. The maceration process is carried out using 1 kg of ketepeng cina leaf simplicia powder with 10 L of 70% ethanol solvent. Substitution of solvent done 2 x 24 hours as much as 6 L. Remaserasi done for 1 x 24 hours with solvent 4 L. Maserat then evaporated with rotary evaporator to get thick extract from leaf ketepeng china. The value of rendement of extract obtained is 15, 039%. The effectiveness of the extraction process is affected by the type of solvent used, the particle size of simplicia, and the duration of the extraction process. Phytochemical screening test of chinese leaf extract can be seen in Table 3. Extracts obtained using reagent showed positive results on flavonoids, tannins, saponins, steroids, alkaloids and phenolics, but negative results of triterpenoids were obtained. The results of phytochemical tests that have been performed have the same results with the results of research that has been done by Jose et al. (2011) to ketepeng cina ethanol extract containing flavonoids, alkaloids, tannins, saponins, steroids and phenolic.

Table 3. Phytochemical profiles of leaf ketepeng cina (Senna alata L.)

Group of compounds	Ethanol Extract	Simplicia
Flavonoids	+	+
Alkaloids	+	+
Triterpenoids	-	-
Steroids	+	+
Saponins	+	+
Tannins	+	+
Phenolic	+	+

Description: (+) identified, (-) unidentified

3.2. Induction of Alloxan in Male Rats

The mice used were 2-3 months old with mouse weights ranging from 150-200 g. Test animals are in good health and not physically disabled. Female mice were not used in this study because the possibility of esterogenic hormone in female mice can affect results and observations, therefore this study only used male rats. Selection of male rats was done to avoid the hormonal effects that can arise in female rats. The estrogen hormone of female rats has a healing effect on pancreatic β cells in the island of Langerhans diabetic rats due to alloxan, where β pancreas has esterogen hormone receptors that cause insulin release. Therefore, this study used only male rats [7].

Blood glucose levels of mice were measured first, this was due to know the blood glucose levels of the rat test before alloxan induced and as evidence that the test rats used did not suffer from DM before and can be used as a comparison on the final result of blood glucose levels after treatment. Rats are swallowed ±18 hours before blood sampling with the aim of avoiding the influence of food. In this study performed fasting blood glucose measurements to determine how much the response of insulin in balancing blood sugar and this process is more effective for the measurement of blood glucose in patients with type 1 diabetes mellitus [8].

Blood samples were taken retroorbitalis plexus from eye veins as much as 0.5-1 cc using hematocrit capillary pipette.[9] Collecting samples using hematocrit capillary pipette through EDTA Vacutainer tube wall so that blood is not lysed, so as not to disturb the results of glucose levels when reading with a spectrophotometer.[10] Serum was taken using a micro pipette inserted into a test tube which had given 1 mL of phenol aminoantipirin glucose oxidase reagent (GOD-PAP). Blood samples obtained, centrifuged at 2500 rpm for 10 minutes. The goal is centrifuged in order to separate the blood constituent components such as plasma and serum.

Samples were incubated for 10 - 15 minutes at a temperature of 20 - 25°C. The goal is because the enzymes glucose oxidase and peroxidase take a certain time to react optimum [11] After obtaining the initial blood glucose levels of the treatment rats (except the normal group), alloxan induced with a dose of 130 mg / kgBW dissolved in intraperitoneal 0.9% NaCl to a blood glucose level of ≥200 mg / dL, with a three-day induction range. The purpose of alloxan induction is that mice can be conditioned DM type 1.

After a lapse of three days after induction of blood glucose levels of mice was seen using a glucose strip by taking a small amount of blood from a rat tail when blood glucose level 200 mg / dL then re-induced alloxan to glucose .≥200 mg / dL. In the span of twice the alloxan induction of rat blood glucose level is still less ≤ 200 mg / dL. This is because the dose used 130 mg / kgBB so that the damage is produced is partial damage of cells β pancreas, so that cells β Langerhans can still produce insulin. Then re-administered alloxan induction dosage of 130 mg / kgBB dissolved with 0.9% NaCl, showed the results of rat blood glucose levels starting to increase up to> 200 mg / dL after 4 - 5x alloxan induction.[12] Comparison of blood glucose levels before and after induced alloxan can be seen in Figure 1.

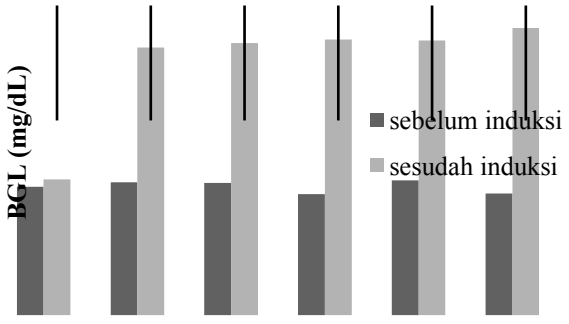

Figure 1. The comparison of BGL before and post induces alloxan.

From the result of paired t-test test between group to blood glucose level, there was significant difference (p <0,05) between group before and after induction that alloksan able to make mouse state hyperglycemia.

3.3. Measurement of Blood Glucose Level

After it was confirmed that mice had diabetes, 24 mice were divided into 6 groups as in Table 1. The groupings were divided according to the range of blood glucose levels after diabetes so that blood glucose levels in one group were not too different. After administration of the test dosage once daily

for 15 days measurements of blood glucose levels performed on days 5, 10, and 15 were measured using a DTN-410-K spectrophotometer. The average result of blood glucose on day 0 to -15 day.

Blood glucose levels of positive control group given insulin therapy with 1 IU / kg BW doses subcutaneously. Selection of insulin therapy as a positive control group was used for comparison with decreased blood glucose levels in other treatment groups where insulin was a therapy used as a type of DM 1[3]. Blood glucose levels in the negative control group continued to increase. A negative control group was given 0.5% NaCMC was used as a comparison of blood glucose levels of the treatment group and positive control group. After 15 days injection of blood glucose level of mice did not decrease blood glucose level because 0,0% NaCMC solution did not affect to decrease blood glucose level causing the response of blood glucose level to alloxan induction decrease phase and increase of blood glucose level (fluctuation) which means experiencing up and down all day and setisp moment depending on the incoming food and Physic activity [13].

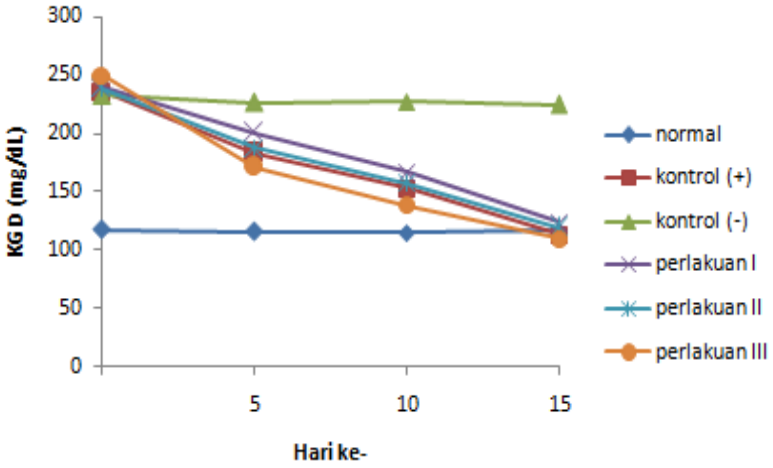

Figure 2. Graph of mouse BGL averages after alloxan induction

Based on the graph above it can be seen that the Group I, II and III have decreased blood glucose levels after administration of extract with doses of 200, 400 and 800 mg / kg BW for 15 days can be seen in figure 2. Can be concluded leaf extract of chinese ketepeng with dose 200 , 400, and 800 mg / kgBW have antidiabetic activity because it can decrease blood glucose level in diabetic mice. The decrease of rat blood glucose level in the treatment group decreased for 15 days after giving extract which showed difference with negative group which was given Na CMC solution which did not have antidiabetic activity.

Decrease in blood glucose levels is caused by the chemical content in chinese leaf ethanol extract that has antidiabetic activity such as flavonoids. Flavonoids have antidiabetic activity with mechanisms significantly increase antioxidant enzyme activity and are able to regenerate damaged pancreatic β cells so that insulin deficiency can be overcome.[14]

3.4. Determination of AUC (Area Under Curve) Value as an Indicator of Blood Glucose Effectivenes

After the average blood glucose level from day 0 to day 15, then calculated the value of area under the curve / Area Under Curve (AUC) to know the change of blood glucose level from day 0 to day 15. Changes in blood glucose levels from each treatment group were known by calculating the AUC value on day 0 to day 15 (AUC_{0-15}). AUC_{0-15} values are inversely proportional to antidiabetic activity. The lower the AUC group of treatments, the better the activity is in the decreased blood glucose level.15 The greater the percentage decrease in glucose levels the better the antidiabetic activity.

The AUC score of positive control group obtained is quite large as well as the treatment groups I, II, and III because in the positive group given insulin as a therapy for decreased blood glucose levels. In the treatment groups I, II, III obtained AUC_{0-15} values respectively 2475.6 and 39.945%; 2618.55

and 47.54%; 2448.425 and 57.72% respectively. The results of calculations AUC_{0-15} and PBGL at doses of 800 mg/kg BW is the best dose because it has the lowest AUC value and percent decrease in blood glucose levels highest than the dose of extract 200 and 400 mg/kgBW.

Flavonoids such as phenolics act as glucosidase enzyme inhibitors. Glucosidase inhibition is a potential agent for DM because the glucosidase enzyme affects biologically relevant processes[14]. Flavonoids can also prevent diabetes by reducing the reductase of aldose and the regeneration of islet cells from the pancreas, as well as increasing the release of insulin and Ca^{2+} uptake[16]. Tannins are known to be astringent that can precipitate the intestinal mucous membrane protein and form a layer that protects the intestine thus inhibiting glucose uptake. In addition there are saponins that have activity as antidiabetes. Saponin is an inhibitor of the α-glucosidase enzyme by entering into the cell membrane to form a more permeable structure thereby increasing the permeability of the small intestine to increase the uptake of the substance so as to inhibit the absorption of nutrient molecules that should be rapidly absorbed such as glucose [17].

ANOVA test results obtained results that have a significant frequency along with Post Hoc LSD test (Smallest Significant Difference). LSDAL post hoc assay as a follow-up test of one way ANOVA, ie to see the truth or amount of data significant to blood glucose levels between groups.18 The LSD post hoc test analysis, it is known that there is significant data on normal levels. each of the other treatment groups ($p < 0.05$). But there was no significant difference between normal group and treatment group III ($p > 0,05$). In the positive group there was no significant difference with treatment group I, II, and III ($p > 0,05$). This shows that chinese leaf ethanol extract works effectively in lowering blood glucose levels. But there was a significant difference between the positive group and the negative group and the normal group ($p < 0.05$). This is due to the positive group decreased blood glucose levels due to insulin administration whereas in the negative and normal group there was no decrease in blood glucose levels. Negative group with treatment group I and II there was no significant difference ($p > 0,05$), whereas in group III there was a significant difference ($p < 0,05$).

3.5. Effective Dose (ED_{50})

According to the Department of Pharmacology and Therapeutic Faculty of Medicine University of Indonesia (2007), 50% effective dose (ED_{50}) is a dose that has therapeutic effect on 50% of individuals (median therapy dose). Calculation of ED_{50} ethanol extract of ketepeng leaf china. The obtained linear equation is $y = 0,029x + 34,85$ (figure 3). This is due to the percentage of blood glucose level decrease of one of the three extracts >50%, so that the dose used is enough to reach the effective dose of 50% of test animals in the decrease of blood glucose level is 522.413 mg/ kgBW.

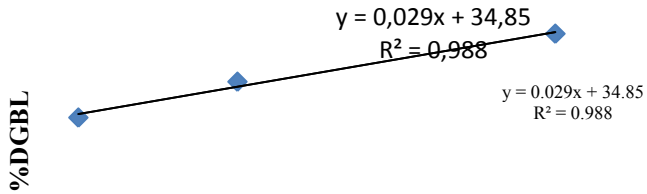

Figure 3. Graph of linear regression between dose and % DBGL of ketepeng cina leaves (Senna alata L.)

4. Conclusion

There is influence of dose difference to% DBGL ethanol extract 70% leaf ketepeng china where in treatment III (800 mg/kgBW) has the highest percentage of decrease in blood glucose level followed by treatment dose II (400 mg/kgBW), then treatment dose I (200 mg/kgBW), which is 39.94%, 47.54%, and 57.72%. This shows the dose of ethanol extract of 70% of the leaf ketepeng china

treatment III (800 mg / kgBW) has the best antidiabetic activity. Effective dose (ED_{50}) extract of ethanol leaves ketepeng china (*Senna alata* L.) that is 522,413 mg/kgBW.

Acknowledgment
Authors would like to express their gratitude toward Sriwijaya University PNBP Sateks ResearchGrant 2018 that made this research possible.

References

[1] Indonesian Ministry of Health 1995 *Materia Medika Indonesia* (Jakarta : Indonesian FDA)
[2] Kaneto, H Yoshitaka K, Jun M, Taka M, Yoshio F, Yutaka U *et al* 1999, Beneficial effects of antioxidants in diabetes: possible protection of pancreatic β–cells against glucose toxicity *Diabetes Journals*, **48**
[3] Indonesian Ministry of Health 2005 *Pharmaceutical care untuk penyakit diabetes mellitus*, Direktorat Bina Farmasi Komunitas dan Klinik (Jakarta, Indonesia)
[4] Harborne, JB 1998 *Metode fitokimia* edisi kedua (Bandung: Penerbit ITB)
[5] Goodman LS and Gilman A 1985 *The Pharmacological basis of therapeutics* (New york : Macmillan). 1941-2015
[6] Tuyet T, Chuyen NV 2007 Antihiperglycemic activity of an aqueous extract from flower buds of *Clistocalyx operculatus* (Roxb.) Merr and Perry *Biosci Biotechnol Biochem* **71**: 69– 76
[7] Smith JB and Mangkoewidjojo, S 1988 *Pemeliharaan, pembiakan, dan penggunaan hewan percobaan di daerah tropis* (Jakarta: Universitas Indonesia Press)
[8] Sharma A, Fish BL, Moulder JE, Medhora M and Baker JE *et al* 2014 *Safety and Blood Sample Volume and Quality of Refined retroorbital Bleeding Technique in Rats Using a Lateral Approach*, Departments of Radiation Oncology, Surgery and Medicine, Medical Collage of Wisconsin, Milwaukee, WI. **43(2)**: 63-66
[9] Kurniati I 2012 Pemberian *platelet rich plasma* topikal meningkatkan proses regenerasi jaringan luka pada tikus putih, *Thesis,* M.Si, Program Studi Biomedik, Universitas Udayana, Denpasar, Indonesia
[10] Yuniarti N, Maulawati RN and Pramono S 2014 Pengaruh pemberian fraksi larut air ekstrak etanol pisang kapas (*Musa paradisiaca* L.) terhadap kadar glukosa darah secara in vivo danpelacakan senyawa aktifnya *Traditional Medicine Journal*, **19(2)**:57
[11] Sujono TA and Sutrisna EM 2010 Pengaruh lama praperlakuan flavonoid rutin terhadap efek hipoglikemik tolbutamid pada tikus jantan yang diinduksi aloksan *Jurnal Penelitian Sains & Teknologi,* **11(2)**: 94 – 98
[12] Lenzen S 2008 The mechanisms of alloxan and streptozotocin induced diabetes *Diabetologia* **51**:216 – 226
[13] Pereira DF, Cazarolli L H, Lavado C, Mengatto V, Guedes A and Pizzolatti M G *et al* 2011 Effects of flavonoids on a-glucosidase activity: Potential targets for glucose homeostasis *Nutrition,***27:** 1161 **-** 1167
[14] Chotimah C, Sutrisna EM, and Wahyuni, AS 2008 Uji penurunan kadar glukosa darah oleh ekstrak air herba jaka tuwa (*Scoparia dulcis* L.) pada kelinci jantan yang dibebani glukosa *Pharmacon* **9(2)**:50
[15] Shandar HK, Kumar B, Pasher S, Tiwari P, Salhan M and Sharma P 2011 A Review of Phytochemistry and Pharmacology of Flavonoids *International Pharmaceutica Sciencia*, **1(1) :** 37
[16] Rotblatt M 2002 *Evidence based herbal medicine* (London: Haney and Bilvus)
[17] Santoso S 2003 *Statistik Deskriptif Konsep dan Aplikasi dengan MS Excel dan SPSS*, Andi, Yogyakarta, Indonesia
[18] Jose S, Usha P T A and Priya PM 2011 Comparative study of ethanolic extract of *Cassia alata* and *Tectona grandis* against pathogenic bacteria. *Res. Phytochem. Pharmacol* **2(1)**:41- 44