Effect of Ethanol Extract Leaf Buasbuas *(Premna pubescens.* Blume) To Decrease Blood Sugar Levels in Rats Male (*Rattus norvegicus*) The Induced Alloxan

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ABSTRACT: This study aimed to analyze the effect of ethanol extract of leaves Buasbuas (*Premnapubescens*. Blume) / EEP to (i) a decrease in blood sugar levels (KGD), and (ii) the increase in body weight (BW) in rats with diabetes mellitus (DM). This type of research is experimentally using completely randomized design (CRD) non factorial using 20 head of white male rats (*Rattusnorvegicus*) Wistar were divided into five groups: (i) negative control / non diabetes mellitus (KN), (ii) positive control (KD), (iii) control drug / group of non DM by EEP 200 mg / kg (KE), (iv) diabetic group by EEP 200 mg / kg (P2), and (v) diabetic group by EEP 300 mg / kg (P3). DM conditions obtained by alloxan induction dose of 150 mg / kg were injected intraperitoneally. KGD measurement is done using a glucometer two days after alloxan induced. Mice with KGD ≥ 200 mg / dl otherwise have diabetes. Then, DM rats (except KD) EEP given every other day for 28 days orally using a needle probe. Making extract using maceration method with 96% ethanol. KGD measurement and BB performed every four days in the morning. Data KGD and BB were analyzed using one-way ANOVA followed by Tukey's test using SPSS 21.0. Results showed EEP dose of 200 mg / kg body weight to lower and raise the BB KGD significantly (P <0.05), but the EEP dose of 300 mg / kg did not give effect to a decrease or increase in BB rats KGD DM. EEP Award dose of 200 mg / kg in mice does not give effect to the KGD or BB.

KEYWORDS: *Premna pubescen* (EEP), alloxan, blood sugar levels, weight

1. INTRODUCTION

Diabetes is one disease that often occurs in today's society. Chairman of the International Diabetes Federation's Asia Fasifik (IDF-WPR) namely, Professor Nam Cho, in the discussion on November 13, 2014, had mentioned that the number of diabetics in Indonesia, putting this country is ranked fifth in the world with a figure of 9.1 million soul (Subarkah, 2014). Mahendra et al (2008) have argued that the treatment of diabetes mellitus and health care have spent substantial funds. The amount of the fee due diabetics should regularly injecting insulin (for patients with type I diabetes mellitus) and administration of oral hypoglycemic agents (for patients with type II DM). When this has been much research done on the potential medicinal plants, and further note that the potency of the drug due to the antioxidant properties owned plants (read: flavonoids). Antioxidants can improve insulin secretion (Winarsi and Purwanto, 2010). Flavonoids as one group of phenolic compounds play a role in preventing cell damage and its cellular components by reactive free radicals (Redha, 2010). Winarsi et al (2013) stated that the flavonoid is able to act as an antidiabetic and antiatherogenik. The unique thing of premna is due to the flavonoid-containing compounds luteolin and apigenin. A number of preclinical studies regarding luteolin had shown that the compound has a wide range of biological activity (Lazaro, 2009).

Content of Metabolites Secondary *Premna pubescens* (Blume)

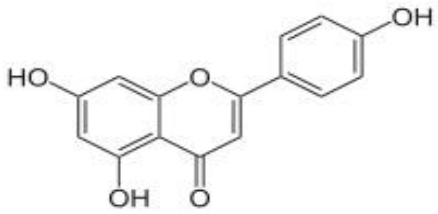
Based on the results Restuati et al (2014) regarding the identification and determination of plants buasbuas referred to in this study (obtained Bogor-based Field Botanical Research Center for Biology LIPI Bogor on July 11, 2012), it can be seen that the Latin name of this plant species isbuasbuas *Premna pubescens* (Blume). From the result of determination, stated that *pubescens Premna* thisbelonging to the family Verbenaceae. The test results of secondary metabolite identificationleaves *Premnapubescens* (Blume) made Restuati et al (2014) using 96% ethanol extract showed that this plant contains alkaloids, flavonoids, saponins and phenolic. For more details, can be seen in Table 2.1 below:

Table 1. Results of phytochemical screeningleaves *Premnapubescens* (Blume)

Indicators	Observations	Secondary Metabolites
Depositionred white and	+	Alkaloids
red orange colored	+	Flavonoids
frothForming	+	Saponin
Color brown	-	Steroid
Tinted bluish green	+	Phenolic

Source: Restuati et al (2014)

From these results, it is also known that levels of apigenin contained in the ethanol extract *Premna pubescens* (Blume) is set at 35, 56 mcg / g (Restuati et al, 2014). Apigenin which is a derivative of these flavonoids have activity as anti hyperglycemic and can reduce levels of cholesterol, LDL and increase HDL in adult Wistar rats (Thiruvenkatasubramaniam and Jayakar, 2010). From the results of research conducted by Husni (2005), obtained data showing the level of antioxidant activity of components luteolin and apigenin which amounted to 74.10% (luteolin) and 58.10% (apigenin).



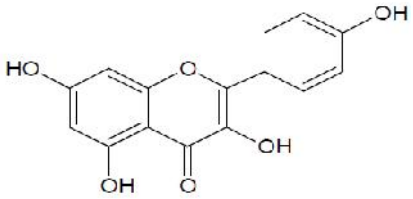


Figure 1. (a) Apigenin **Figure 1. (b) luteolin**

Source: Sastrohamidjojo (1996)

2. METHODS

Location and Time Research

This research was conducted at the Chemical Laboratory Animals of the Faculty of Mathematics and Natural Sciences, University of Medan (UNIMED), Biology Laboratory UNIMED and Laboratory of Pathology and Anatomy University of North Sumatra. This research was conducted in January to June 2016.

Population and Sample

Population in this study were male rats Wistar strain obtained from the Laboratory of Pharmacy University of North Sumatra. The sample consisted of 20 rats was 2 months old with a mean body weight - average 150-250 grams.

Equipment and Materials Research

tools used consisted of a blender, stock bottles, filter paper, funnel, spatula, sample bottles, jerrycans, *rotary* evaporator, *refrigerator,*equipment / surgical instruments, syringes, needles, measuring cups, microtomes, glass objects , *cover*glass, cage maintenance, husks, wire netting, scales, oral sonde, glucometers, and needle frank.

The materials used in this study are the leaves buasbuas, 96% ethanol, 0.9% NaCl, water, white male rats, feed (pellets) C551, alloxan, CMC 1%.

Preparation and Procurement Cage Rat

Cage used plastic rectangular shape with a size of 40 x 20 x 15 cm. Each cage was placed on a rat's tail. Cages were given pedestal chaff from the sawdust with a thickness of ± 0.5 cm - 1 cm to absorb urine, which are replaced every day.

Acclimatization Rats

Acclimatizationin this study were performed for 7 days (one week) before starting treatment. White mice were fed with pellet type C551 and drink every day at 08.00 am in the morning. The amount of feed to be administered per rat tail with a range of 10% of the weight of the mice. While the number given excessive drinking in 500 ml bottles. Total food and drink were left each day is measured.

Preparation and Determination of Dose Giving Leaf Ethanol Extract Buasbuas *(Premna pubescens*. Blume) /

(EEP).

EEP making procedure refers to Restuati (2015), ie by extraction maceration method by ethanol 96%. The leaves are used for the manufacture of EEP as much as 6 kg (wet weight) and dried until it reaches a total of 1.2 kg of dry weight. Once macerated, acquired EEP (in the form of pasta) 164 gr so unknown percentage of marinade sebesar13,7%. Buasbuas leaf ethanol extract (EEP) was administered orally to the mice with CMC was dissolved in 1%. EEP is given to a concentration of 4%, then the volume of CMC were added to the EEP is determined using the following formula:

$$\text{ml Larutan CMC} = \frac{\text{dosis EEP (gr)}\ x\ \text{BB (gr)}}{1000} x \frac{100}{\text{konsentrasi EEP}}$$

Preparation Solution and Determination of Dose Alloxan

Preparation of alloxan refers the procedures performed by Prasetiawan (2015). Making short procedure as follows: weigh the powder alloxan then reconstituted with NaCl 0.9%. Alloxan was given a concentration of 3%, then the volume of NaCl is added to dissolve the alloxan also use the above formula.

Treatment

This studyconsisted of five groups, consisting of 3 control group and two treatment groups. The extract for the treatment given after experiencing hyperglycemic mice and diabetes. Giving extract refers Prasetiawan (2015) which is conducted once a day for 28 days after the mice developed diabetes mellitus.

Table 2. Grouping of experimental animals*(Rattus*norvegicus)

Notation	Group
KN	Feed + drinking
KE	Feed+ drinking + EEP (200 mg / kg bw)
KD	drinking + alloxan Feed
P2	Feed + drinking + EEP alloxan (200 mg / kg bw)
P3	feed + drinking + EEP alloxan (300 mg / kg bw)

Measurement Weight

weight of mice was measured using OHAUS scales to the nearest 0.1 gram. Body weight of rats will be weighed once every 4 days until the end of the study.

Measurement of Blood Glucose

Measurement of blood sugar levels refers to Suarsana et al (2010). Blood glucose levels were determined by biosensor glucose oxidase method, using a measuring device digital blood sugar (glucometers). Mice with blood glucose levels above 200 mg / dl^3 was diagnosed with diabetes mellitus. Measurement of blood sugar levels is done every morning with an interval of 4 days (Uray, 2009; Prasetiawan, 2015). The first measurement done on day 0 (after rats became diabetic and alloxan induced), then performed once every 4 days for 28 days, bringing the total number of observations as much as 8 times.

Design of Experiments

This research includes an experimental study using a completely randomized design (CRD) non factorial.

Analysis Techniques Data

Quantitative Datawere analyzed using analysis of variance (ANOVA) in one direction with a significance level $\alpha = 0.05$. If the test results showed no significant differences / highly significant ($P < 0.05$) then continued by *Least Significant Difference* (LSD) or least significant difference (LSD) to see the significance of the results obtained and comparison of each treatment. Data analysis was performed using *the Statistical Software Product and Service Solutions* (SPSS) version 21.0.

3. RESULTS AND DISCUSSION

Effect of EEP Against Rat Weight

Based on the Analysis of Variance to the effects of a dose of EEP at different doses in rats with diabetes mellitus, acquired that dose EEP significant effect on body weight of rats ($F = 8.884$; $P = 0.000$). Effect of EEP can be seen from the graph below:

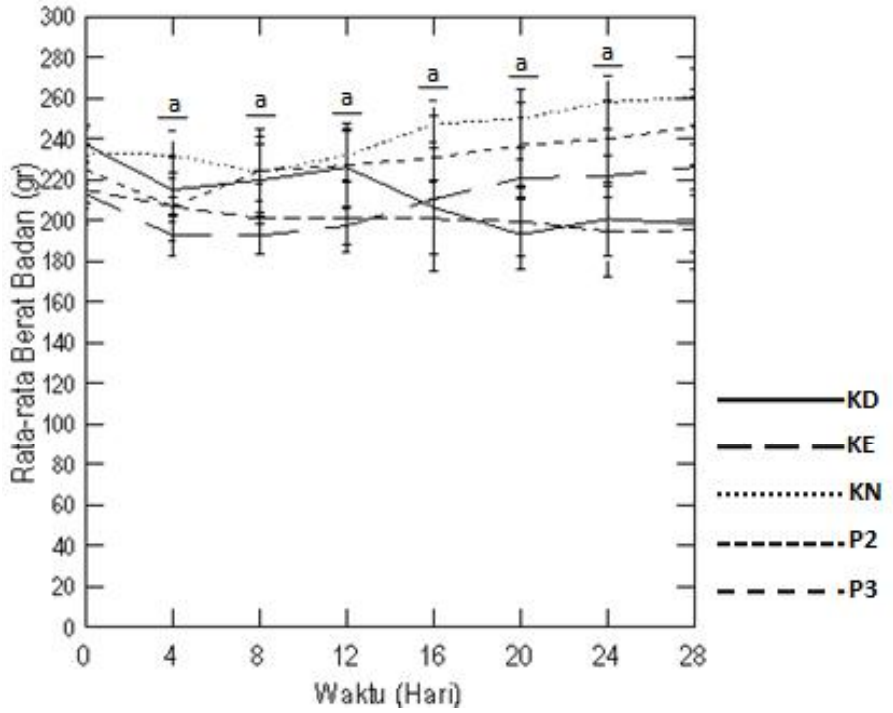


Figure 2. **Changes in body weight in mice treated per four days of observation.** KD = DM; KE = Non DM + EEP (200 mg / kg); KN = Negative Control; P2 = DM + EEP 200 mg / kg; P3 = DM + EEP 300 mg / kg. , The same letter at the time (days) the same observations on the diagram shows not significantly different (Tukey test)

Rat body weight KN group, P2 and KE seen an increase during the treatment, while the KD group and P3 seems to experience weight loss. Weight gain after a diabetic condition experienced group of P2 showed the effect of EEP, which EEP is able to reduce the effects of the diabetic condition with increased body weight in group P2. The mechanism of reduction of the effects of diabetes by EEP EEP allegedly because it contains flavonoids are able to act like insulin, so give good influence for the conditions of hyperglycemia in diabetics. As stated by velayutham *et*al.(2013) that flavonoids appear to regulate the digestion of carbohydrates, insulin secretion, insulin signaling, and improve glucose uptake in tissues that depend on insulin through a variety of intracellular signaling pathways. EEP Award at a dose of 300 mg / kg did not reduce the effects of diabetes (weight reduction). Hormesis law states that herbs (in this case the ethanol extract of the leaves buasbuas) with an improved dosing not necessarily give better results. Sometimes with fewer doses can give a better impact. In this study, a dose of 200 mg / kg body weight provides better results for weight gain in diabetic rats diabandingkan dose administration of 300 mg / kg.

Effect of EEP Against Diabetes Blood Sugar Mice

Based on the Analysis of Variance to the effects of a dose of EEP at different doses in rats with diabetes mellitus, acquired that dose KGD EEP significant effect on rats (F = 68.349; P = 0.000). Effect of EEP can be seen from the graph below:

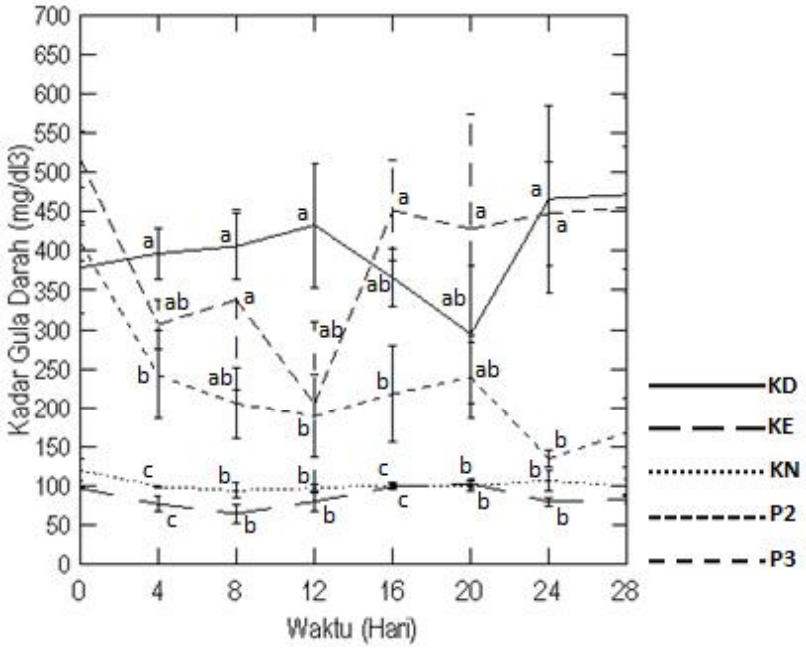


Figure 3. **Changes in blood sugar levels in mice treated per four days of observation.** KD = DM; KE = Non DM + EEP (200 mg / kg); KN = Negative Control; P2 = DM + EEP 200 mg / kg; P3 = DM + EEP 300 mg / kg. Different letters at the time (days) the same observations on the diagram indicates significantly different (Tukey test).

In the treatment group, effect given EEP group P2 and P3 are also different. In the P2 group KGD visible deterioration from the beginning until the end of the observation. The decrease was caused by the presence of flavonoids in particular apigenin and luteolin as well as saponins contained in EEP. All three of these compounds have been reported to have anti-diabetic and anti-hyperglycemic properties (hypoglycemic) (Lazaro. 2009; Thiruvenkatasubramaniam and Jayakar.2010; Wresdiyati *et*al.,2015). The mechanism of the decline in KGD has been described by velayutham *et al* (2013) that flavonoids increase the secretion of insulin, controlling hyperglycemia through the regulation of glucose metabolism in hepatocytes, reducing insulin resistance, inflammation and oxidative stress in muscle and fat, and increase the uptake of glucose in skeletal muscle and adipose tissue. Furthermore, it was reported that saponins (one of the active compounds in EEP) able to prevent an increase in the absorption of glucose in the small intestine through penginaktivasian enzymes that play a role in the utilization of glucose (Smith and Andanlawo, 2012), one like the inhibition of the enzyme α-glucosidase in the intestine by flavonoids (Pereira *et al,*2011). Inhibition of these enzymes work will delay the process of breakdown and absorption of glucose in themembrane of *brush border* the small intestine which will indirectly suppress the increase in blood glucose levels (Bosenberg & Zyl, 2008). Furthermore, from these results is known that EEP at a dose of 200 mg / kg give better effect to decrease KGD EEP diabetic rats compared with a dose of 300 mg / kg. Differences hasilini allegedly influenced by several factors, such as differences in the severity of hyperglycemia experienced by the two groups who initially blood sugar levels are higher than P2 P3. Furthermore, the law Hormesis.

4. CONCLUSION

GivingThe ethanol extract of leaves buasbuas*(Premnapubescens.* Blume) / EEP at a dose of 200 mg / kg effect on weight gain in alloxan-induced diabetic rats. EEP Award dose of 200 mg / kg effect on blood sugar levels decrease alloxan-induced diabetic rats.

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