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EFFECT OF DUKU (*Lansium domesticum Corr*) SEED EXTRACT FOR URINATION ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Duku seed extract has hypoglycemic effect. The result of phytochemical screening showed the presence of flavonoid, triterpen and saponin. The aims of study are to determine of effect duku seed extract to urination on alloxan induced diabetic rats. Experimental study with pre-and post-test with control group design was carried out at Laboratory Chemistry Analysis MIPA Faculty and Animal House at Faculty of Medicine Sriwijaya University University in January 2014 to March 2014. The study subject were 30 male white rats 2-aged 3 months and weight 200-250 g. Rats were made diabetes by intraperitoneal administration of 160 mg/kg of alloxan. All rats divided into five groups, K1 which is negative control were treated with aquadest, K2 treated with duku seed extract 100mg/kgbw, K3 treated with duku seed extract 200mg/kgbw, K4 treated with duku seed extract 300mg/kgbw, and K5 treated with glimepiride 0,018mg/kgbw at single dose per day for a period of 14 days. The data obtained was analyzed with normality test, paired sample t test, ANOVA, and Post Hoc test. Data of blood glucose, body weight, and urine volume have normally distribution. All subject has blood glucose >200mg/dL Duku seed extract and glimepiride can decrease urine volume in alloxan-induced diabetes rats. Duku seed extract dose 100 mg/kgbw were no significant difference ($p < 0.05$) between glimepiride to decreased volume urine on 11th and 15thdays.It can be concluded that low dose of duku seed extract can be used to decreased urine volume on alloxan induced diabetic rats.

Keywords: *Lansium domesticum*, urine, volume, alloxan

1. INTRODUCTION

Diabetes mellitus (DM) is one of the biggest threats to global health. The number of DM sufferers in the world has increased, in 2000 as many as 175.4 million, in 2010 as many as 279.3 million and in 2020 it is estimated that as many as 300 million.[1] A survey conducted by the World Health Organization (WHO) shows that Indonesia has a prevalence of diabetes mellitus of 8.6% of the total population. Indonesia ranks 9th in the estimation of the world's diabetes mellitus epidemiology in 2010 with 7 million cases and will continue to rise to 5th in 2030 with 20 million cases. In South Sumatra, diabetes mellitus is a non-communicable disease with the second highest prevalence, per 28.85 per 1000 in 2009.[2] Diabetes mellitus is a metabolic disease characterized by hyperglycemia. Hyperglycemic conditions according to can result in the formation of reactive oxygen species (ROS = Reactive Oxygen Species).[3] Excessive ROS can cause oxidative stress and can cause damage to pancreatic B cells.[4] The second cause is

insulin resistance, the amount of insulin is sufficient but the insulin is not sensitive so it is not able to work optimally and glucose cannot enter the cells which results in the use of glucose as energy being inhibited and causing a lack of energy in the cells. The third cause is the result of a combination of the two causes.[5]

Clinical symptoms that accompany people with diabetes mellitus include polyuria (frequent urination), polydipsia (lots of drinking) and polyphagia (a lot of eating). This weight loss occurs due to the loss of fat.[6] Loss of fat due to lipolysis causes hyperlipidemia so that fat loss occurs quickly.[7] If these symptoms are not treated and last a long time, they can lead to long-term complications, such as atherosclerosis of the heart, legs and brain, peripheral nerve damage, retinal disorders and kidney damage.[8]

The most important DM therapy is food therapy by adjusting the patient's diet in collaboration with nutritionists to determine what foods can be consumed. Drugs can be given if food therapy does not work.[9] Indonesia is a tropical country that is rich in natural resources. Forest diversity as a natural wealth still needs to be explored and known for its potential as a source of medicinal ingredients including antidiabetic[11]. In accordance with the priority of research, development and application of health science and technology in the field of medicine and medical devices for the period 2005-2025, including standardized herbal products and phytopharmaceuticals.[12] One of Indonesia's native plants which is thought to have anti-diabetic potential is the duku plant (Lansium domesticum Corr) which is a native plant of Indonesia, especially in the South Sumatra region. Chemical compounds contained in the Lansium domesticum Corr plant are tetranorterpenoids, namely domesticulide AE, limonoids, namely andirobin derivatives, Lansium domesticum Corr plants are metal angolensates, mexicanoid, azadiradione, onoceranoid and dukunolide, and ferpenoids. The aims of study are to determine of effect duku seed extract to urination on alloxan induced diabetic rats.

2. METHOD

This study is an experimental study with a pre and post test design with a control group design. The control group was used as a comparison. The negative control was placebo and the positive control was glimepiride. This research was conducted in February – March 2014 at the Laboratory of Organic Chemistry, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sriwijaya University and Experimental Laboratory of Pharmacology and Animal House, Faculty of Medicine, Sriwijaya University. The test animals used were adult male wistar rats obtained from the Bandung Institute of Technology (ITB). Mice were taken from a homogeneous population aged 2-3 months and weighing 200-250 grams. The total number of samples is 30 samples with the number of each group as many as 6 samples with the number of treatments 5 treatments. The research variables were rat urine volume and duku seed extract (dosage 100, 200, 300 mg/kgbw). Data analysis used Kolmogorov Smirnov test, paired sample t test, independent sample t test and one way ANOVA test.

3. RESULT

This qualitative phytochemical test uses analytical techniques with color visualization.

Table 1. Phytochemical Qualitative Test Results of Duku Seed Extract

No	Parameter		Result	Analysis Techniques
1.	Alkaloid	Wagner	Negative	
		Mayer	Negative	
		Dragenndorf	Negative	
	Steroid		Negative	Visualization
2.	Flavonoid		Positive	Color
3.	Tanin		Negative	
4.	Saponin		Positive	
5.	Triterpenoid		Positive	
6.	Hidroquinon		Negative	

Test Results Certificate No. : 405.001/LPSB IPB/11/14

Characteristics of Research Samples by Group. In the research subjects Rattus norweglcus rats were measured body weight (BB), blood glucose levels before being induced and given treatment as well as measuring urine volume before being induced and given treatment. The results of the urine volume measurement are the primary data of the study which were statistically analyzed using SPSS version 20. The results of the analysis showed that the weight of the rats in this study was normally distributed because the p value > 0.05 can be seen in the table below this.

Table 2. Rat Body Weight Normality Test Before Treatment

Variable	Aquadest	EBD 100mg/kgbb	EBD 200mg/kgbb	EBD 300mg/kgbb	Glimepiride	*p value*
BB (gram)	218.33 ±4.08	218.33 ±4.08	218.33 ±4.08	218.33 ±4.08	218.33 ±4.08	0.215

Levene test p=0.05. Description EBD: duku seed extract, BB: body weight

Changes in Urine Volume of Each Group Up to 24 hours

Changes in urine volume for each group until the 24th hour showed that in the group treated with duku seed extract at doses of 100 mg/kg, 200 mg/kg, 300 mg/kg and the aquadest group, there was an increase in urine volume at each measurement time. Except for the dose group of 100 mg/kg at

the 12th hour there was a decrease in urine volume. All groups experienced a fairly high increase in urine volume at 24 hours. Meanwhile, in the positive control group given glimepiride, urine volume increased at 24 hours.Changes in Urine Volume of Each Group Until Day 15. Changes in urine volume for each group until the 15th day showed that on the 3rd day after being given treatment, the duku seed extract group at a dose of 100 mg/kgbw and 200 mg/kgbw decreased urine volume, while the duku seed extract group at a dose of 300 mg/kg kgbw and the glimepiride group tended to have an increase in urine volume. Furthermore, on day 7 to day 15 all treatment groups experienced a decrease in urine volume.The following table also presents the decrease in urine volume after being given the test preparation for 14 days.

Table 2. Differences in Urine Volume of Each Group on Day 0 to Day 15

Treatment group	Urine volume before treatment (ml) Day 0	Urine volume before treatment (ml) 15th day	Difference (ml) (Δ)
Aquadest	20.96	18.23	2.73
EBD 100 mg/kgbb	21.93	7.05	14.88
EBD 200 mg/kgbb	32.91	6.93	25.98
EBD 300 mg/kgbb	27.10	14.66	12.44
Glimepiride	24.76	12.85	11.91

To assess the effectiveness of Lansium domesticum Corr seed extract in reducing urine volume in each treatment group until the 15th day, a paired sample t-test was performed. on the 3rd, 7th, 11th and 15th day of each group which can be seen in table 7. From table 7 it can be concluded that in the negative control group there was no difference in urine volume before and after being given aquadest. In the 100 and 200 mg/kgbw duku seed extracts, there were differences in urine volume from the 3rd day to the 15th day after treatment. Meanwhile, in the positive control group who were given glimepiride after 15 days of treatment, there was a difference in urine volume. Likewise, the group that was given duku seed extract 300 mg/kgbw after 15 days of treatment, there was a difference in urine volume.

Table 3. Urine Volume of Each Group on Day 0 to Day 15

Observation time	Treatment group		Urine volume (ml) before treatment	Urine volume before (ml) treatment	*p value*
Day 3	Aquadest		20.96±2.72	18.80±5.11	0.416
	EBD mg/kgbb	100	21.93±5.39	8.5±3.75	**0.014**
	EBD mg/kgbb	200	32.91±17.71	27.70±15.44	**0.004**
	EBD mg/kgbb	300	27.10±3.65	37.10±24.81	0.362
	Glimepiride		24.76±7.41	31.43±11.47	0.326
Day 7	Aquadest		20.96±2.72	15.50±6.30	0.499
	EBD mg/kgbb	100	21.93±5.39	5.60±1.33	**0.002**
	EBD mg/kgbb	200	32.91±17.71	13.3±7.83	**0.005**
	EBD mg/kgbb	300	27.10±3.65	23.23±16.88	0.896
	Glimepiride		24.76±7.41	16.06±6.71	0.436
Day 11	Aquadest		20.96±2.72	15.50±6.30	0.185
	EBD mg/kgbb	100	21.93±5.39	5.60±1.33	**0.001**
	EBD mg/kgbb	200	32.91±17.71	13.3±7.83	**0.005**
	EBD mg/kgbb	300	27.10±3.65	23.23±16.88	0.060
	Glimepiride		24.76±7.41	16.06±6.71	0.090
Day 15	Aquadest		20.96±2.72	18.23±4.21	0.308
	EBD mg/kgbb	100	21.93±5.39	7.05±2.88	**0.001**
	EBD mg/kgbb	200	32.91±17.71	6.93±2.76	**0.009**
	EBD mg/kgbb	300	27.10±3.65	14.66±7.62	**0.027**
	Glimepiride		24.76±7.41	12.85±4.71	**0.024**

Paired sample t-test, p = 0.05

Description : EBD: Duku Seed Extract, SD: Standard Deviation

To determine the suitability of the dose of duku seed extract to urine volume, a post-hoc statistical test was carried out to see whether there was a difference in urine volume levels between the two groups. The results of the analysis can be seen in table 4 below.

Table 4. Dosage Conformity Test for Aquades-Duku Seed Extract-Glimepiride Group Each Time Observation with Post-Hoc Test

Observation time	Treatment group	*p value*			
		Day 3	Day 7	Day 11	Day 15
Aquadest	EBD 100 mg/kgbb	0.020	0.089	0.056	0.004
	EBD 200 mg/kgbb	0.680	0.988	0.981	0.004
	EBD 300 mg/kgbb	0.470	0.851	0.823	1.000
	Glimepiride	0.205	0,942	1.000	0.623
EBD 100mg/kgbb	Aquadest	0.020	0.089	0.056	0.004
	EBD 200 mg/kgbb	0.130	0.340	0.254	1.000
	EBD 300 mg/kgbb	0.163	0.325	0.214	0.106
	Glimepiride	0.019	0.012	0.058	0.458
EBD 200mg/kgbb	Aquadest	0.680	0.988	0.981	0.004
	EBD 100 mg/kgbb	0.130	0.340	0.254	1.000
	EBD 300 mg/kgbb	0.927	0.950	0.694	0.096
	Glimepiride	0.988	1.000	0.961	0.419
EBD 300mg/kgbb	Aquadest	0.470	0.851	0.823	1.000
	EBD 100 mg/kgbb	0.163	0.325	0.214	0.106
	EBD 200 mg/kgbb	0.927	0.950	0.694	0.096
	Glimepiride	0.984	1.000	0.860	1.000
Glimepiride	Aquadest	0.205	0.942	1.000	0.023
	EBD 100 mg/kgbb	0.019	0.012	0.058	0.458
	EBD 200 mg/kgbb	0.988	1.000	0.961	0.419
	EBD 300 mg/kgbb	0.984	0.949	0.860	1.000

Post-hoc test, p=0.05

From table 4 it can be concluded that on the 11th and 15th days, the doses of 100 and 200 mg/kgbw of duku seed extract and glimepiride were different from the group treated with aquadest. Therefore, it is necessary to do an unpaired t-test to see which dose of 100 mg/kgbw and 200 mg/kgbw of duku seed extract is more effective in reducing urine volume or has effectiveness in compatibility with glimepiride. From the results of the analysis using the unpaired t-test, it can be concluded that on day 3 to day 15 there is a difference between the urine volume in the group given duku seed extract at a dose of 100 mg/kgBW and the group given glimepiride. Duku seed extract at a dose of 200 mg/kgbw only showed a difference in urine volume with the group given glimepiride on the 15th day.

4. DISCUSSION

The urine output recorded during the process of diabetes induction was seen to increase in each group of diabetic rats compared to the aquadest group. Increased urine production (polyuria) may be due to glycosuria that arises where glucose is an osmotic diuretic so that diuresis is greatly increased with the loss of various electrolytes. This increase in urine output is also caused by more active kidney work to control the concentration of glucose in the blood. When the concentration of glucose in the blood increases , then the kidneys are increasingly active in filtering glucose and removing the glucose through the urine. Therefore, rats suffering from diabetes will often excrete large volumes of urine.[14]

Based on the results of data analysis, it can be concluded that the proposed hypothesis (Ho) is accepted, namely that there is no significant difference in effectiveness between duku seed extract (L.domesticum) and glimepiride in reducing urine volume (Rattus norvegicus) in alloxan-induced diabetes. Urine is the residual fluid excreted by the kidneys which is then removed from the body through the process of urination. Urinary excretion is needed to get rid of residual molecules in the blood that are filtered by the kidneys and to maintain body fluid homeostasis. Urine is filtered in the kidneys, carried through the ureters to the bladder, finally excreted out of the body through the urethra. Urine consists of water with dissolved materials in the form of metabolic wastes such as urea, dissolved salts, and organic matter. Urine-forming fluids and materials come from the blood or interstitial fluid. The composition of urine changes during the reabsorption process as molecules important to the body, such as glucose, are reabsorbed into the body via carrier molecules. The remaining fluid contains high levels of urea and various excess or potentially toxic compounds that will be flushed out of the body. The material contained in the urine can be known through urinalysis. Hyperglycemia causes an increase in glucose filtration by the kidney glomerulus so that the kidney threshold is missed.[15] F This leads to high excretion of glucose into the urine (glucosuria) thereby inducing an osmotic diuresis which causes the patient to urinate a lot which can lead to dehydration. Dehydration causes hyperosmolarity due to increased concentrations of blood glucose and interstitial fluid. from the thirst center in the brain, this condition encourages sufferers to drink a lot.[16]

In diabetic rats there is also a diuresis event, where spending a lot of water through the urine will cause an increase in sodium that is excreted with the urine. This is also caused by the occurrence of hyperactivity of a-adrenoceptors, on glomerular afferents or the occurrence of systemic arteriolar blockage of adrenaline levels in vascular dysfunction which plays a major role in the occurrence of glomerular hypertension caused by hyperfiltration.[17] the process of glucosuria, hyperglycemia, hypoinsulinemia, polydipsia, polyphagia, weight loss.[18] Due to the occurrence of hyperglycemia during diabetes, it causes high levels of glucose in the blood, also the food eaten every day which is a source of glucose will always increase the concentration of glucose in the blood. High levels of glucose in the blood can return the nephrons so that they can exceed the threshold for the absorption of glucose in the urine[19]. the administration of duku seed extract at a dose of 100 mg/kgbb. This is also thought to be due to the content of several

groups of secondary metabolites found in the duku seed extract. The group of compounds thought to play a role are flavonoids, triterpenoids and saponins.

A good diuretic effect can be related to the content of flavonoids. Flavonoids can increase the concentration of Na + and K + in the urine. There are many relationships between urine volume and concentration of Na+, this aspect related to the mechanism of action of diuretic drugs is to decrease tubular reabsorption of these ions.[20] Several flavonoids or plant extracts with high flavonoid content have also been reported to cause potential inhibition of renal glucose reabsorption through inhibition of the sodium glucose symporter located in the proximal renal tubule.[21] research by[22], showed that saponins can stimulate ATPase and decrease ANP, so that it can reduce urine volume. Where the mechanism of action is opposite to that of furosemide, namely by modulating Na ATPase. Furosemide works by increasing the excretion of Na+, K+ and Cr1- by inhibiting the reabsorption of Na+, K+ and C1- in the loop of Henle, so that urine volume increases. While the triterpenoid compounds actually have a mechanism of action similar to furosemide, namely by increasing the volume of urine by decreasing the active transport of Na+, where the activity of Na+, K+ ATPase is inhibited25.

5. CONCLUSION

Lansium domesticum Corr seed extract has the effect of reducing urine volume in alloxan-induced diabetic rats. There was a significant difference in urine volume in the group of rats given Lansium domesticum Corr extract at a dose of 100 mg/kgbw on days -3, 7,11 and 15. The group of active substances thought to play a role in reducing urine volume in diabetic rats induced by alloxan are flavonoids, saponins, and triterpenoids.

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