

Activity Test Of Salam Extract (*Syzygium polyanthum*) On Diabetes Disease Using White Rats (*Ratus Novergicus*)

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Abstract

*Diabetes Mellitus is a chronic metabolic disease characterized by elevated blood sugar levels due to impaired secretion or action of insulin. The use of herbal plants such as as salam leaves (*syzygium polyanthum*) is one of the alternative treatment methods. This study aims to evaluate the effectiveness and optimal dosage of salam leaf extract in lowering blood glucose levels in male rats (*Rattus Novergicus*) induced by alloxan. A total of 30 rats we divided into six groups: normal control, negative control, positive control (metformin), and three treatment groups with extract doses of 200, 300, and 400 Mg/KgBB. Blood glucose levels were measured on days 15,20,25, and 30. Data were analyzed using ANOVA with a significance level of 95%. The results show that salam leaf extract significantly lowers blood glucose levels, with the most effective dose being 400 Mg/KgBB. This study supports the potential of salam leaves as an effective, safe, and affordable natural antidiabetic agent***Keywords:** *Maximum of 5 keywords separated by commas, (according to the scope of articles and journals). [Times New Roman 9 single spaced font, and italic]*

Keywords: Salam Leaf, *Syzygium Polyanthum*, Diabetes Mellitus, Blood Glucose, White Rats, Alloxan

Abstrak

Diabetes Melitus merupakan penyakit metabolik kronis yang ditandai dengan peningkatan kadar gula darah akibat gangguan sekresi atau kerja insulin. Penggunaan tumbuhan herbal seperti daun salam (*syzygium polyanthum*) adalah salah satu metode pengobatan alternatif. Penelitian ini bertujuan untuk mengevaluasi efektivitas dan dosis optimal ekstrak daun salam dalam menurunkan kadar glukosa darah pada tikus Jantan (*Rattus Novergicus*) yang diinduksi aloksan. Sebanyak 30 tikus dibagi dalam enam kelompok: kontrol normal, kontrol negative, kontrol positif (metformin), serta tiga kelompok perlakuan dengan dosis ekstrak 200, 300, dan 400 Mg/KgBB. Kadar glukosa darah diukur pada hari ke-15, 20, 25, dan 30. Data dianalisis menggunakan ANOVA dengan tingkat signifikan 95%. Hasil menunjukkan bahwa ekstrak daun salam secara signifikan menurunkan kadar glukosa darah, dengan dosis paling efektif yaitu 400 Mg/KgBB. Penelitian ini mendukung potensi daun salam sebagai agen antidiabetes alami yang efektif, aman, dan terjangkau.

Kata kunci: Daun Salam, *Syzygium polyanthum*, Diabetes Melitus, Glukosa Darah, Tikus Putih, Aloksan.

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia due to impaired secretion or action of insulin. Chronic hyperglycemia can cause long-term complications in vital organs, including the eyes, kidneys, nerves, and heart. Type 2 diabetes mellitus is generally characterized by insulin resistance, while type 1 diabetes mellitus is caused by autoimmune damage to the pancreatic β cells. This disorder inhibits glucose from entering the cells optimally, thereby increasing blood glucose levels. In addition to systemic complications, diabetes mellitus is also prone to secondary infections, such as diabetic ulcers caused by the bacterium *Staphylococcus epidermis*.

Diabetes mellitus is a global health issue with a continuously increasing prevalence. World Health Organization recorded 422 million cases with 1,5 million deaths each year, and the International Diabetes Federation estimates that this number will reach 783 million by 2045. In Indonesia, particularly in North Sumatra, there were 249,519 cases of diabetes mellitus in 2019, but only 57,92% received healthcare services.

METHODOLOGY

The type of this research is a pure experiment, testing the effect of ethanol extract of salam leaves (*syzygium polyanthum*) on the reduction of blood glucose levels in male white rats, with metformin as a comparison.

The design of this study is a pre-test and post-test control group design, where blood glucose measurements in rats are conducted before and after treatment.

The sample used in this study is salam leaves (*syzygium polyanthum*) found on Jl. Nusa Indah Raya, Komplek Pembda Tk1 Tanjung Sari, Medan, North Sumatra. The samples taken were tested purposively, meaning the samples were taken without considering the location and place of the samples. This research was conducted at the integrated Laboratory of Universitas Prima Indonesia, Medan.

Tools and Materials

The tools used in this research are blenders, analytical scales, rotary evaporator, pipettes, beakers, glucometer, water bath, gloves, and other laboratory equipment. The materials used in this study are salam leaves (*syzygium polyanthum*), alloxan, pellets, aquadest, 96% ethanol, parchment paper, filter paper, metformin, test animals, syringes, sulfuric acid, chloral hydrate, hydrochloric acid, Mg Powder, ethyl acetate, HCL, acetic acid, mayer's, dragendorff's, bouchardat's and sampel extract.

Preparing the sample

Sample preparation begins with collecting fresh salam leaves and cleaning them, then washing them with running water. The next step is to dry the salam leaves using a drying cabinet, and after the leaves are dry, they are ground using a blender. The next step is to maceration the sample using 96% ethanol and soak it for 3 days. After that, the salam leaf extract is filtered using filter paper, then the solvent is evaporated using a rotary evaporator at a temperature of 60°C.

The yield of the extract is calculated using the formula:

$$\text{Yield (\%)} = (\text{Weight of extract obtained} / \text{Weight of dry simplicia}) \times 100\%$$

Preparing of Test Animals

In this study, male white rats (*rattus novergicus*) aged 3 months with an initial weight of 100-200 grams were used. Active movement indicates healthy rats. Before the experiment, the mice were adapted for 1 week. The mice were placed in polypropylene cages in a maintenance room set at a temperature of 24°C and relative humidity of 60-70%. The rats were given pellets daily and drinking water as they pleased.

Diabetes Induction and Treatment Administration

To create diabetic mice, alloxan was used to damage the pancreatic beta cells for 14 days. Starting with measuring the body weight of the rats, the using a glucometer to measure the blood glucose levels of the rats. The dose of alloxan given was 30 Mg/kg body weight administered to the rats intraperitoneally, and they were given 10% glucose to drink for two days before being replaced with plain water. The glucose level is measured with a glucometer, and it is considered diabetes if the rat has a blood sugar level > 160mg/d

Table 1. Division of Experimental Groups

Group	Administration Dosage
1	Normal Control
2	Negative Control (-) : aquades
3	Positive Control (+) : Metformin 4,5mg/kg body weight
4	EEDS 200 mg/Kg body weight
5	EEDS 300 mg/Kg body weight
6	EEDS 400 mg/Kg body weight

Note: EEDS = Ethanol Extract of Salam Leaves

The estimated dose using the human to rat conversion factor (0.018) , for rats weighing 200 g. The dose of alloxan is 0.54 Mg per rat (30 Mg x 0.018). The dose for salam leaf extract is

3.6 Mg (200 Mg/Kg), 5.4 (300 Mg/Kg),

and 7.2 (400 Mg/Kg). The extract is given orally for 15 days via a gastric tube.

Blood Sugar Level Measurement

After 30 days, the rats were treated according to their respective groups, and then each rat was observed on days 15, 20, 25 and 30 to measure their blood glucose levels using the glucose meter strip test method. Data were obtained from the results of the glucometer observations.

Data Analysis

The data obtained were analyzed using One-Way ANOVA to determine the differences between groups, followed by post-hoc tests and independent T-tests. The statistical result for this study were conducted using the

SPSS version 27 (Windows) application with a confidence level of 95%. The result data are present in the form of graphs and tables.

RESULT AND DISCUSSION

Data Analysis

The plant used in this study is the Salam leaf (*Syzygium polyanthum*), which has been identified at the Laboratory of Universitas Prima Indonesia.

Yield Results

Salam leaves are extracted by collecting 5 Kg of leaves, then cleaning, washing, drying, and grinding them. The leaves were ground into a powder, and then maceration for 3 days using 5 liters of 96% ethanol as a solvent. Then it was concentrated using a rotary evaporator, yielding 138 grams of a thick brown extract. The yield obtained from fresh salam leaves to obtain extract was 27.6%, a figure that meets the requirements of the Farmakope Herbal Indonesia, which is no less than 18.2% (Farmakope Herbal Indonesia.)

Results of Phytochemical Screening Test

Phytochemical screening shows that the ethanol extract of Salam

Table 2. Phytochemical Screening Show That The Ethanol of Salam Leaves (*Syzygium polyanthum*)

No	Phytochemical Test	Results	sign
1	Flavonoid	Positif	+
2	Tanin	Positif	+
3	Saponin	Positif	+
4	Alkaloid	Positif	+
6	Triterpenoid/Steroid	Positif	+

Note: (+) = contains a group of secondary metabolite compounds

Blood Sugar Level Test From this research, which was conducted on the effectiveness test of ethanol extract of salam leaves on male white rats, the results of blood glucose level in the male rats were obtained as shown in the following table 3.

Table 3. Results of The Average Glucose Levels of Mice

Treatment	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Normal control	100	98	100	101	95	94	96
Negative control	106	142	168	213	235	248	262
Positive control	111	132	159	178	132	123	108
EEDS 200Mg/Kg Body weight	114	163	202	243	204	155	142
EEDS 300Mg/Kg Body weight	105	137	164	197	177	158	140
EEDS 400Mg/Kg Body weight	104	171	216	240	210	164	130

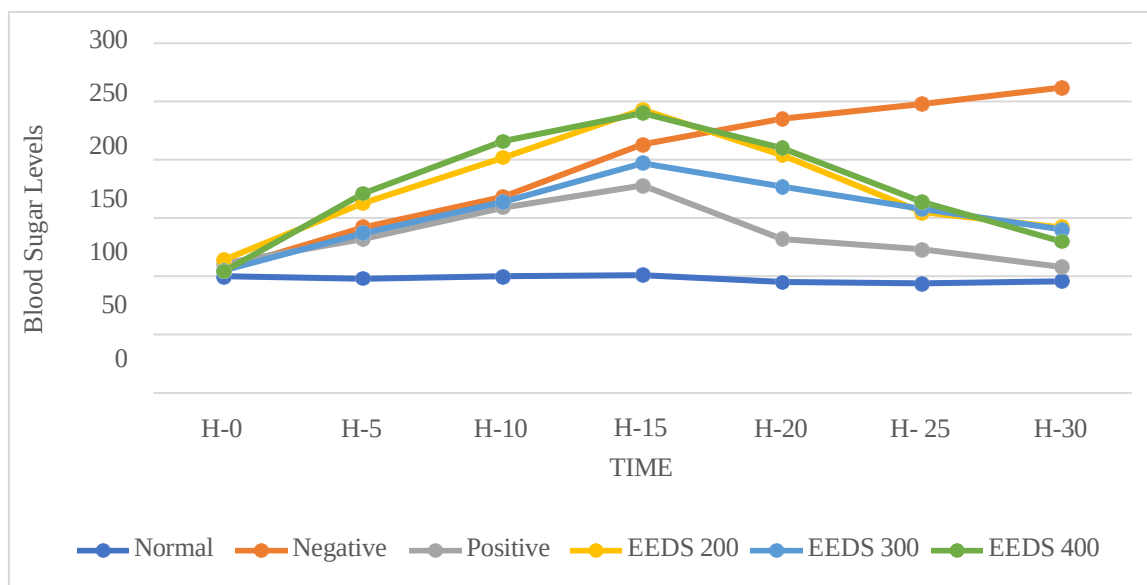


Figure 1. Graph of The Average Results of Measuring Mouse Blood Glucose Levels

The test salam leaf (*Syzygium polyanthum*) resulted in a significant decrease in blood glucose levels in white rats induced with alloxan. In 30 days, group K-IV (200 Mg/Kg BW) experienced a decrease from 243 Mg/dL to 142 Mg/dL, K-V (300 Mg/Kg BW) from 197 Mg/dL to 140 Mg/dL, and K-VI (400 Mg/Kg BW) from 240 Mg/dL to 130 Mg/dL. The decrease in the blood sugar levels of the rats indicates a hypoglycemic effect of the salam leaf extract comparable to the negative control K-II, and is comparable to the efficacy of the positive control metformin K-III.

Data Analysis

Data analysis was conducted using One-Way ANOVA technique in SPSS with Shapiro-wilk test to check the data normality. The result of the analysis showed.

Table 4. Shapiro-Wilk Test Results

Mean ± SD							
Treatment	N	KN	KP	EEDS 200mg/kg BB H20	EEDS 300mg/kg BB H25	EEDS 400mg/kg BB H30	P-Value 0,000
Induksi Aloksan	101,20 ± 15,23	* 212,80 ± 108,11	* 177,80 ± 18,95	* 242,60 ± 37,81	* 196,60 ± 11,71	* 239,60 ± 93,98	0,014
EEDS 200mg/k gBB Hari 20	95,00 ± 9,91	* 235,00 ± 97,47	* 131,60 ± 4,74	* 204,00 ± 46,28	* 177,40 ± 11,48	* 209,80 ± 102,22	0,012
EEDS 300mg/k gBB Hari 25	93,80 ± 14,82	* 247,60 ± 94,267	* 122,60 ± 2,60	* 155,20 ± 45,20	* 158,20 ± 14,97	* 164,00 ± 66,44	0,003
EEDS 400mg/k	96,20 ±	* 262,20 ±	* 108,40 ±	* 142,40 ±	* 140,20 ±	* 130,20 ±	0,000

gBB Hari	9,65	92,21	7,63	36,50	17,13	41,48
30						

From the data table above, significant results ($P < 0.005$) were shown on the 25th and 30th days after the administration of EEDS at doses of 30 Mg/Kg BW and 400 Mg/Kg BW. To identify group treatments, a Post Hoc LSD analysis was used to show significant differences.

Note:

- 1 = Significant difference compared to the normal group (K-I) with ($P < 0.005$)
- 2 = Significant difference compared to the negative control group (K-II) with ($P < 0.005$)
- 3 = Significant difference compared to the positive control group (K-III) with ($P < 0.005$)
- 4 = Significant difference compared to the dose of 200 Mg/KgBW (K-IV) with ($P < 0.005$)
- 5 = Significant difference compared to the dose of 300 Mg/KgBW (K-V) with ($P < 0.005$)
- 6 = Significant difference compared to the dose of 400 Mg/KgBW (K-VI) with ($P < 0.005$)

Discussion

Diabetes mellitus is a disease where the increase in the patient's blood sugar indicates insufficient secretion of the insulin hormone or no insulin in the pancreas, with or without insulin effect disturbances.

The effectiveness of salam leaf extract (*Syzygium polyanthum*) in lowering blood sugar levels can be explained by its bioactive compounds such as flavonoids, tannins, and alkaloids, which play a role in antidiabetic activity. Flavonoids are one of the groups of compounds that can lower blood glucose levels in the body. The mechanism of flavonoids as hypoglycemics is thought to inhibit the reabsorption of glucose from the kidneys and can increase the solubility of blood glucose, making it easier to excrete through urine. Salam leaves (*Syzygium polyanthum*) have the ability to act as an astringent that can precipitate mucus membrane proteins and form a layer that protects the intestines, thereby inhibiting glucose absorption, which results in a decrease in blood glucose levels.

Tannin has the ability to provide protection by acting as a free radical scavenger and activating antioxidant enzymes. Tannin also has the ability to enhance glucose uptake through insulin signaling pathway mediators such as activation of PI3K, P38 MAPK, and GLUT-4 translocation. The combination of these compounds contributes to the reduction of blood sugar levels through various mechanisms.

In the conducted research, it was shown that administering higher doses of ethanol extract salam leaves, such as 400 Mg/Kg BW, had a more significant effect in lowering blood sugar levels. These studies not only support the proposed mechanism but also highlight the effectiveness of using natural ingredients like salam leaves as complementary therapy.

Based on the analysis results and available literature I assess that a 400 Mg/Kg BW dose of salam leaf extract is the best choice for lowering blood sugar levels in diabetic rats. Its effect, which is close to that of the positive control, indicates that this dose has high efficacy without showing significant fluctuations. Furthermore, these results demonstrate the great potential of local herbal plants as a source of active ingredients for the treatment of chronic diseases such as diabetes.

CONCLUSION

Based on the research on the activity test of salam leaf extract (*Syzygium polyanthum*) against diabetes using white rats (*Rattus norvegicus*), it can be concluded that salam leaf extract (*Syzygium polyanthum*) significantly reduces the blood glucose levels of diabetic rats. From the statistical analysis with a value ($p < 0.005$), which means a significant reduction in blood glucose levels in all observations, the extract of salam leaves (*Syzygium polyanthum*) significantly reduced the blood glucose levels of rats at a dose of 400 Mg/Kg Bw compared to doses of 200 Mg/Kg Bw and 300 Mg/KgBw.

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