The effect of Leaf Salam Extracts (*Syzygium polyanthum*) in diabetes mellitus therapy on wistar albino rats

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**Abstract.** Diabetes mellitus (DM) is one of the chronic disease that caused the most complications and even death. Non-Insulin Dependent Diabetes Mellitus (NIDDM) is the most widely recognized. It is closely related with the incidence of insulin resistance. This research is an in vivo experimental study with post-test control group design located in laboratory of animal house and biomolecular laboratory Faculty of Medicine, Universitas Sriwijaya, Indonesia. The subjects of this study were albino rats (*Rattus norvegicus*), Wistar strain, divided into 5 groups are negative control, positive control (TZD), Leaf Salam Extract (LSE) 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW. Rat was injected by Human Insulin (ip) 1.8 IU/kgBW and orally glucose for 14 days. In this study it was found that there was no distinction in blood sugar levels in the positive control group with LSE 200 mg/kgBW, LSE 100 mg/kgBW and LSE 50 mg/kgBW and there were no difference in insulin levels in the positive control group with LSE 200 mg/kgBW and LSE 100 mg/kgBW and LSE 50 mg/kgBW. So, it can be concluded that LSE (*Syzygium polyanthum*) was effective in lowering blood sugar and insulin levels of Wistar albino rat induced diabetes mellitus.

1. **Introduction**

Diabetes mellitus (DM) is one of the most chronic diseases that cause complications and even death. Non-Insulin Dependent Diabetes Mellitus (NIDDM) is the most widely recognized [1]. WHO predicts the number of DM sufferers is quite large in the coming years, Indonesia is now ranked fourth after the United States, China and India, there will be an increase in prevalence from 8.4 million (2000) to around 21.3 million (2030) [2].

NIDDM is related to insulin resistance, which is insulin produced but cannot be attached to its receptor. An important mechanism in managing DM is stimulating uptake glucose from plasma into peripheral tissues, namely muscle and adipose tissue. To enter the cell, glucose penetrates the cell membrane through a facilitated diffusion mechanism using a carrier protein known as glucose transporter/GLUT4 [3].
Management of diabetes usually requires a long time, even a lifetime, and not only includes pharmacological therapies, but also non-pharmacological such as diet and exercise. There are many types of oral anti-diabetic, but considering the use of these drugs is long-term, efforts to develop effective drugs with raw materials that are cheap and easy to get and close to the physiology of the body continue to be developed [4].

Based on the previous study, the methanol extract of salam leaf diminished blood sugar levels in hyperglycemia-induced rats by restraining glucose ingestion in the digestive system and by increasing uptake of glucose in peripheral tissues [5].

This research will hopefully be able to explain scientifically how the effects of salam leaf extract in reducing blood sugar levels in Wistar albino rats so that later this salam leaf extract can be developed into Standard Herbal Medicine (OHT) even to Fitofarmaka and can be used by humans as one of the drugs of choice for Diabetes Mellitus.

2. Methods
This research is an in vivo experimental study with post-test control group design located in laboratory of animal house and biomolecular laboratory Faculty of Medicine, Universitas Sriwijaya, Indonesia.

The subjects of this study were albino rats (Rattus norvegicus), Wistar strain, divided into 5 groups are negative control (Aquadest), positive control (TZD), Leaf Salam Extract (LSE) 50 mg/kgBW, 100 mg/kgBW and 200 mg/kgBW. Rat was injected by Human Insulin (ip) with a dose of 1,8 IU/kgBW and orally glucose (drink water) for 14 days [6].

Simplicia are obtained from medicinal plants in the Yogyakarta area. Then simplicia was macerated with 90% ethanol solvent for 72 hours with stirring. Macerate is then filtered and the filtrate obtained is concentrated with a rotary evaporator to get a thick extract of salam leaves.

The parameters examined include blood sugar levels (peripheral vein, Glucometer) and insulin (peripheral vein, ELISA). Examination of blood sugar and insulin levels was carried out 3 times, namely pre-duction, pre-treatment and post-treatment.

3. Results
After induction with Human Insulin (ip) with a dose of 1,8 IU/kgBW and orally glucose for 14 days, blood sugar and insulin levels were monitored. After 14 days of induction, blood sugar levels in all groups are increase significantly (p<0.05).

Table 1. Blood sugar level before and after human insulin induction.

| Group             | Before            | After            | p value |
|-------------------|-------------------|-------------------|---------|
| Negative Control  | 90,17 ± 6,27      | 126,67 ± 3,777    | 0,000<sup>a</sup> |
| Positive Control  | 90,17 ± 6,27      | 126,67 ± 2,503    | 0,000<sup>a</sup> |
| LSE 50 mg/kgBW    | 90,17 ± 6,27      | 126,67 ± 2,041    | 0,000<sup>a</sup> |
| LSE 100 mg/kgBW   | 90,17 ± 6,27      | 126,67 ± 2,503    | 0,000<sup>a</sup> |
| LSE 200 mg/kgBW   | 90,17 ± 6,27      | 126,67 ± 1,472    | 0,000<sup>a</sup> |

<sup>a</sup>Paired T Test

As well as insulin levels found significant differences after 14 days of Human Insulin induction.

Table 2. Insulin level before and after human insulin induction.

| Group             | Before            | After            | p value |
|-------------------|-------------------|-------------------|---------|
| Negative Control  | 0,66 ± 0,039      | 0,93 ± 0,053      | 0,000<sup>a</sup> |
| Positive Control  | 0,66 ± 0,039      | 0,93 ± 0,035      | 0,000<sup>a</sup> |
| LSE 50 mg/kgBW    | 0,66 ± 0,039      | 0,93 ± 0,023      | 0,028<sup>b</sup> |
| LSE 100 mg/kgBW   | 0,66 ± 0,039      | 0,93 ± 0,028      | 0,028<sup>b</sup> |
| LSE 200 mg/kgBW   | 0,66 ± 0,039      | 0,93 ± 0,031      | 0,000<sup>a</sup> |

<sup>a</sup>Paired T Test
<sup>b</sup>Wilcoxon Test
3.1. Blood sugar level

3.1.1. Effectiveness of salam leaf extract on blood sugar levels.
Blood sugar levels of each group were re-checked after 14 days of treatment. There was a significant decrease in blood sugar levels in positive control group (p = 0.000), LSE 50 mg/kgBW (p = 0.006), LSE 100 mg/kgBW (p = 0.001) and LSE 200 mg/kgBW (p = 0.000), while for the negative control group there was no significant distinction in blood sugar levels before and after treatment (p = 0.880). The highest decrease in blood sugar levels was found in the positive control group followed by LSE 200 mg/kgBW, LSE 100 mg/kgBW and LSE 50 mg/kgBW sequentially.

Table 3. Blood sugar level before and after salam leaf extract induction.

| Group                  | Before        | After         | p value |
|------------------------|---------------|---------------|---------|
| Negative Control       | 126.67 ± 3,777| 126.50 ± 2,665| 0.880a  |
| Positive Control       | 126.67 ± 2,503| 95.00 ± 3,225 | 0.000a  |
| LSE 50 mg/kgBW         | 126.67 ± 2,041| 116.67 ± 5,574| 0.006a  |
| LSE 100 mg/kgBW        | 126.67 ± 2,503| 111.67 ± 5,609| 0.001a  |
| LSE 200 mg/kgBW        | 126.67 ± 1,472| 98.83 ± 4,665 | 0.000a  |

*Paired T Test

3.1.2. Comparison of Effectiveness Of Salam Leaf Extract On Blood Sugar Levels.
Blood sugar levels between groups were then compared. By conformity test using Post hoc showed that there was no distinction in blood sugar levels in the positive control group with LSE 200mg/kgBW, LSE 100 mg/kgBW and LSE 50mg/kgBW.

Table 4. Conformity test of blood sugar level after salam leaf extract induction.

| Group | Negative | Positive | LSE 50 | LSE 100 | LSE 200 |
|-------|----------|----------|--------|---------|---------|
| Negative | 0,004 | 0,011 | 0,001 | 0,030 |
| Positive | 0,004 | 0,992 | 0,745 | 0,483 |
| LSE 50 | 0,011 | 0,992 | 0,483 |
| LSE 100 | 0,001 | 0,935 | 0,492 |
| LSE 200 | 0,030 | 0,921 |

*Post Hoc Test

3.2. Insulin level

3.2.1. Effectiveness of salam leaf extract on blood insulin levels.
Insulin levels of each group were re-checked after 14 days of treatment. There was a significant decrease insulin levels in positive control group (p = 0.000), LSE 50 mg/kgBW (p = 0.000), LSE 100 mg/kgBW (p = 0.001) and LSE 200 mg/kgBW (p = 0.000), while for the negative control group there was no significant distinction in insulin levels before and after treatment (p = 0.672). The highest decrease in insulin levels was found in the positive control group followed by LSE 200 mg/kgBW, LSE 100 mg/kgBW and LSE 50 mg/kgBW sequentially.

3.2.2. Comparison of effectiveness of salam leaf extract on insulin levels.
Insulin levels between groups were then compared. By conformity test using Post hoc demonstrated that there were no difference in insulin levels in the positive control group with LSE 200 mg/kgBW and LSE 100 mg/kgBW and LSE 50 mg/kgBW. Futhermore, there were no difference in insulin levels in the negative control group with LSE 50 mg/kgBW.

4. Discussions
Salam leaf extract (*Syzygium polyanthum*) doses of 50 mg, 100 mg and 200 mg has been appeared to reduce blood sugar levels. In contrast to research conducted in previous study, the optimum dose of
100 mg/kgBW of salam leaf extract can reduce blood sugar levels, in this study the effective dose of salam leaf extract is 50 mg/kg BW which can reduce blood sugar levels up to 8% of blood sugar levels before intervention and with high dose 200 mg/kgBW can reduce blood sugar levels up to 22% in 14 days [7]. This calculation is obtained from table 3 where the before and after result was turned into ratio.

Table 5. Insulin level before and after salam leaf extract induction.

| Group               | Before   | After    | p value |
|---------------------|----------|----------|---------|
| Negative Control    | 0.93 ± 0.053 | 0.919 ± 0.486 | 0.672<sup>a</sup> |
| Positive Control    | 0.93 ± 0.035 | 0.685 ± 0.047 | 0.000<sup>a</sup> |
| LSE 50 mg/kgBW      | 0.93 ± 0.023 | 0.857 ± 0.018 | 0.000<sup>a</sup> |
| LSE 100 mg/kgBW     | 0.93 ± 0.028 | 0.726 ± 0.055 | 0.001<sup>a</sup> |
| LSE 200 mg/kgBW     | 0.93 ± 0.031 | 0.708 ± 0.022 | 0.000<sup>a</sup> |

<sup>a</sup>Paired T Test

Table 6. Conformity test of insulin level after salam leaf extract induction.

|           | Negative | Positive | LSE 50 | LSE 100 | LSE 200 |
|-----------|----------|----------|--------|---------|---------|
| Negative  | 0,000    | 0,000    | 0,096  | 0,000   | 0,000   |
| Positive  | 0,000    | 0,000    | 0,000  | 0,429   | 0,871   |
| LSE 50    | 0,096    | 0,000    | 0,000  | 0,000   | 0,000   |
| LSE 100   | 0,000    | 0,429    | 0,000  | 0,934   |         |
| LSE 200   | 0,000    | 0,871    | 0,000  | 0,934   |         |

<sup>a</sup>Post Hoc Test

In addition to lowering blood sugar levels, Salam leaf extract dose of 50 mg, 100 mg and 200 mg was also proven to reduce insulin levels in hyperglycemic rats models with a minimum dose of 50 mg/kgBW can reduce insulin levels to 7.9% insulin before intervention and with high dose 200 mg/kgBW can reduce insulin levels up to 24% in 14 days.

In 2011, a study was conducted in 65 patients with NIDDM. Patients continued to consume a routine diet and ADO drugs regularly with supplementation of 2 grams of Salam leaf powder for 4 weeks. The results obtained were a decrease in blood sugar levels, total cholesterol, LDL and triglycerides accompanied by HDL increase. Salam leaf has a polyphenol compounds which have an effect on insulin sensitivity, glucose uptake and antioxidants so that it is thought to reduce blood sugar levels. This compound is found in vegetables, fruits and most herbs [8].

The active segment of the salam leaves is likely a polyphenol, water soluble, since more than 80% of the in vitro insulin potentiating movement was expelled by polyvinylpyrrolidone, which ties aromatic hydroxyl groups [9,10]. However, this study does not identify the exact components of LSE thus the active ingredients cannot be investigated yet.

5. Conclusions

Salam leaf extract has a significant effect in reducing blood sugar and insulin levels in the blood. Therefore, considering that this plant is broadly accessible in Indonesia, this plant has the potential to be developed as herbal medicine for Diabetes mellitus.

6. References

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