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FRUIT EXTRACT OF VANILLA (*Vanilla planifolia* Andrews) LOWERS TOTAL BLOOD GLUCOSE IN ALLOXAN-INDUCED HYPERGLYCEMIC MICE

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ABSTRACT

Hyperglycemia, a high level of blood glucose, is a main indicator of diabetes mellitus. Diabetic patients that do not manage the sugar levels in their blood can suffer from many severe complications. One of plant origin bioactive suspected to have anti-hyperglycemic activity is vanillin. This study aimed to determine whether ethanol fruit extract vanilla (*Vanilla planifolia* Andrews), the main source of vanillin, can be used as anti-hyperglycemia in mice induced by alloxan (150mg/kg). Male mice (n=24) were divided into six groups. Group 1 (normal control) did not receive induction or treatment. Group-2 (negative control) was induced with alloxan but received no drug. Group-3 (positive control) was treated with glibenclamide at the dose of 0.039 mg/kg. Whereas group-4, 5 dan 6 were given vanilla extract of 2.1, 4.2, dan 8.4 mg/g body weight respectively. The treatment were given once daily for 14 days. Blood glucose levels were examined on day-0 (base line), 6th, 13th and 20th. The results showed by day-13 all levels of vanilla extract have significantly reduce blood glucose levels compared with negative control group. On the 20th day the effect of reducing blood sugar by vanilla fruit extract has even surpassed the glibenclamide. It can be concluded that ethanol fruit extract of vanilla is potential to be used as antidiabetes.

KEYWORDS: Alloxan, Antihyperglycemia, Diabetes mellitus, Hyperglycemia, vanillin, *Vanilla planifolia*.**I. INTRODUCTION**

Diabetes mellitus is a symptom that arises in a person caused by an increase in blood glucose levels so that it can reduce progressive insulin secretion due to insulin resistance (Soegondo *et al.*, 2010). Diabetes is a dangerous disease for the people of Indonesia. Diabetes occurs because of a lack of insulin, insulin is a substance produced by the pancreas to process blood sugar (glucose) so that it can become energy. In diabetes sugar in the blood cannot be processed into normal energy, even the levels will continue to increase. These events are called hyperglycemia, which is the accumulation of glucose that occurs in the blood. DM can occur due to not meeting insulin as needed or insulin produced is ineffective so that high bloodglucose levels occur .

Vanilla plants (*Vanilla planifolia* Andrews) contains flavonoids and phenol components. Flavonoids are active ingredients of natural ingredients that have been studied to have hypoglycemic activity. Flavonoids also have an inhibitory effect on the enzyme alpha glucosidase through hydroxylation bonds and substitution on the β ring. The principle of inhibition is similar to a drug for handling *diabetes mellitus* which can inhibit the metabolism of sucrose into glucose and fructose (Shanmugavali, 2009). Vanilla plants that contain flavonoids are found in the fruit and vanilla seeds,

besides vanilla also has antioxidant and anti-inflammatory compounds (Srikanth *et al.*, 2012).

Based on the above, the researcher interested in conducting further research to determine the effect of ethanol extract of vanilla beans and multilevel dose difference ethanol extract of vanilla beans to a decrease in total blood glucose levels in mice induced alloxan. This research It is hoped that it can become a new source of information for the community about the efficacy of vanilla fruit so that it can be utilized more optimally. The purpose of this study was to see the effectiveness of vanilla fruit ethanol extract in reducing blood glucose levels in mice and to see the effect on body weight of mice induced by alloxan. The results of this study are expected to provide information and scientific references to the community about the benefits of ethanol extract (*Vanilla planifolia* Andrews) toward to decrease in blood glucose levels as an alternative treatment of patients hyperglycaemia.

II. MATERIAL AND METHODS**Vanilla fruit and extraction**

Vanilla fruits were obtained from a vanilla farmer in Sub District of Talangpadang, the District of Tanggamus, Lampung, Indonesia. The vanilla pods were dried at 40°C for five days, and then sliced up to the thickness of

0.2-0.5 cm. The pieces of pods were macerated using 60% ethanol. Maceration was run for 24 hours and after filtration the maceration was continued and lasted for three days at a temperature range of 20-30°C. Macerate then were evaporated using rotary evaporator at 60°C.

Experimental Design and Treatment

Male mice aged 3-4 months with a body weight ranged 30-40 g used in this study were obtained from Lampung Veterinary Center, Labuhan Ratu, Bandar Lampung, Indonesia. After acclimatization for one week, by using completely randomized design, the test mice (n=24) were grouped into six as follows.

1. Group 1 as normal control group (K₀) are mice that were received nothing except for standard diet.
2. Group 2 as negative control group (K-) are mice that were induced with 150mg/kg alloxan.
3. Group 3 as positive control group (K+) are mice that were induced with alloxan treated with glibenclamid at the doses of 0.039 mg/kg.
4. Group 4, were alloxan-induced mice that received ethanol fruit extract of vanilla at the doses 2.1 mg/g.
5. Group 5, were alloxan-induced mice treated with extract of vanilla at the doses 4.2 mg/g.
6. Group 6, were alloxan-induced mice treated with vanilla extract at the doses 8.4 mg/g.

Alloxan inducement was done subcutaneously 3 times for 6 days. Standard drug of diabetes—the glibenclamide and vanilla extract were given orally using stomach tube everyday for 14 days.

Study parameters

Parameters assessed in this study are body weight and blood glucose levels. Blood sample were taken from tail-tip of the mice. Glucose levels were measured using Glucometer and Easy Touch Blood Glucose Test Strips. Body weight measurement and blood glucose level examination were done before alloxan inducement (baseline) and on day 6, 13 and 20.

Data analysis

The data obtained were analyzed using one-way ANOVA of the SPSS version 15 software, followed by LSD for post hoc test. Both ANOVA and LSD used $\alpha=0.05$ as the significant level.

III. RESULTS AND DISCUSSION

Table 1 showed effects of vanilla fruit extract on body weight of mice before (baseline) and after treatment. Effects of vanilla fruit extract on blood glucose level of mice before inducement and treatment are presented in Table 2.

Table 1: Body weight of mice before and after alloxan-inducement and treatments.

| Treatment | Body weight of mice in g (Mean \pm SEM) | | | |
|--------------------|--|------------------------------|-------------------------------|-------------------------------|
| | Baseline | Day 6 | Day 13 | Day 20 |
| Normal control | 31.0 \pm 1.00 ^a | 32.0 \pm 0.81 ^a | 32.7 \pm 1.03 ^a | 34.5 \pm 0.95 ^a |
| Negative control | 36.7 \pm 1.93 ^a | 35.7 \pm 0.47 ^a | 39.7 \pm 1.10 ^b | 40.2 \pm 0.85 ^b |
| Positive control | 31.7 \pm 1.65 ^a | 33.2 \pm 2.17 ^a | 38.2 \pm 2.42 ^{ab} | 36.7 \pm 2.68 ^{ab} |
| Vanilla 2.1 mg/10g | 32.2 \pm 1.54 ^a | 34.0 \pm 0.70 ^a | 37.7 \pm 1.54 ^a | 37.0 \pm 1.35 ^{ab} |
| Vanilla 4.2 mg/10g | 33.0 \pm 0.91 ^a | 36.7 \pm 1.18 ^a | 36.5 \pm 0.95 ^a | 38.2 \pm 1.25 ^{ab} |
| Vanilla 8.4 mg/10g | 35.0 \pm 0.70 ^a | 32.7 \pm 1.43 ^a | 37.0 \pm 1.41 ^{ab} | 39.0 \pm 2.08 ^{ab} |
| Sig. | 0, 117 | 0,0 27 | 0,0 21 | 0, 235 |

values in the same column followed by the same superscript are not different statistically by LSD test at $\alpha < 0.05$

It is clear from Table 1 that only negative control mice showed a significant body weight compared to the normal group. This figure shows that the effects of alloxan treatment on the increase of body weight. The mechanism of action of alloxan which can selectively

inhibit insulin secretion (insulin resistance) causes glucose to function as the main energy source in the body unable to enter the cell and be carried away by the bloodstream and finally the body uses fat and protein reserves as an energy source (Ewenighi *et al.*, 2015)

Table 2: Blood glucose levels of mice before and after alloxan-inducement and treatments.

| Treatment | Blood glucose levels in mg/dL (Mean \pm SEM) | | | |
|--------------------|---|--------------------------------|---------------------------------|--------------------------------|
| | Baseline | Day 6 | Day 13 | Day 20 |
| Normal control | 70.2 \pm 6.65 ^a | 77.5 \pm 4.09 ^a | 78.2 \pm 5.66 ^a | 100.7 \pm 3.83 ^{ab} |
| Negative control | 84.5 \pm 8.65 ^a | 261.0 \pm 5.30 ^b | 263.2 \pm 4.71 ^d | 268.2 \pm 4.53 ^c |
| Positive control | 82.7 \pm 7.11 ^a | 264.7 \pm 21.05 ^b | 122.0 \pm 6.91 ^b | 93.5 \pm 6.07 ^{ab} |
| Vanilla 2.1 mg/10g | 94.0 \pm 7.15 ^a | 238.2 \pm 4.25 ^b | 169.5 \pm 19.25 ^c | 109.7 \pm 11.83 ^b |
| Vanilla 4.2 mg/10g | 73.5 \pm 12.6 ^a | 250.7 \pm 12.22 ^b | 155.5 \pm 18.41 ^{bc} | 84.25 \pm 5.34 ^a |
| Vanilla 8.4 mg/10g | 80.5 \pm 5.31 ^a | 241.2 \pm 9.10 ^b | 138.7 \pm 6.94 ^{bc} | 80.0 \pm 7.67 ^a |
| Sig. | 0.420 | 0.000 | 0.000 | 0.000 |

values in the same column followed by the same superscript are not different statistically by LSD test at $\alpha < 0.05$

Based on data in Table 2 it can be assumed that ethanol fruit extract of vanilla is effective in reducing blood glucose levels of mice.

From the results of the study it was known that the glibenclamide treatment group (the positive control) could reduce the mean fasting blood glucose level by $93,5 \pm 6.07$ mg/dL was still classified as normal. Glibenclamide is an oral antidiabetic derivative of sulfonylurea which can reduce blood glucose levels affected by diabetes. Decreased blood glucose levels that occur after administration of sulfonylurea are caused by stimulation of insulin secretion from the pancreas. The nature of this stimulation is different from stimulation by glucose because it turns out that when hyperglycemia fails to stimulate adequate secretion of insulin, these drugs are still able to stimulate insulin secretion at high doses (Tony and Suharto, 2005).

In the vanilla treatment group of highest dosage can reduce fasting blood glucose levels by $80,0 \pm 7.67$ mg/dL is still classified as normal levels, from these results it can be seen that the dose of vanilla fruit extract 8.4 mg/10g body weight is more effective in reducing fasting blood glucose levels which have been induced by alloxan compared to moderate doses and low doses. Because the larger the dose given, the greater the decrease in blood glucose levels is caused by chemical compounds contained in vanilla fruits such as vanillin, antioxidants, flavonoids, and phenols in counteracting free radicals.

The effect of reducing blood glucose levels in mice was caused by the presence of bioactive compounds contained in the ethanol extract of vanilla fruit, namely flavonoids, antioxidants, and phenols. These compounds act as antihyperglycemic agents. According to Robertson *et al.*, (2015) antioxidants have been shown to reduce the deteriorating state of hyperglycemic conditions by increasing the mechanism of the immune system.

IV. CONCLUSION

It is clearly illustrated from the data of this study that vanilla fruit extract is as effective as the glibenclamid drug in reducing blood sugar levels. Therefore, it can be concluded that ethanol fruit extract of vanilla is potential to be used as antidiabetes.

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