The activity of isolate from ethyl acetate fraction of mahkota dewa (Phaleria macrocarpa [Scheff.] Boerl) fruits on insulin sensitivity in hyperglycaemic mouse

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Abstract

An experiment has been done to evaluate the activity of isolate from ethyl acetate fraction of mahkota dewa (Phaleria macrocarpa [Scheff.] Boerl) fruits (PM-IIAy1) on insulin sensitivity in hyperglycaemic mouse. The exam was in 7 days and divided into 4 groups, those were normal control, negative control, positive control, and the test group. All mice, except normal control, were fed 5.35 g/kg BW of glucose, then positive control were treated with 143 mg/kg BW of metformin and the test group with 143 mg/kg BW of PM-IIAy1. On the 8th day, after fasted, all mice were injected intravenously 1 g/kg BW of glucose and 0.25 U/kg BW of exogenous insulin, then measured of their blood glucose every 3 minutes during 18 minutes. The result of the experiment showed that PM-IIAy1 could increase insulin sensitivity appeared in capability of insulin in decreasing of blood glucose level significantly (α=0.05) that compared with negative control.

Keywords: Isolate ethyl acetate, insulin sensitivity, Mahkota dewa (Phaleria macrocarpa [Scheff.] Boerl) fruits.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). Sarah Wild et al. (2004) published the prevalence estimation of diabetes in Indonesia that the total number of people with diabetes is projected to rise from 8.4 million in 2000 to 21.3 million in 2030.

Type 2 of diabetes mellitus is the common major form which results from defect insulin, almost always with a major contribution from insulin resistance (Soegondo, 2007). Insulin resistance is an adverse of insulin sensitivity and defined as a reduced ability of insulin to exert its expected metabolic action that is normal biologic effect in blood glucose level (Schteingart, 2005; Tran et al., 2003).

The ways to increase insulin sensitivity include diet, exercise, and drugs such as metformin, can clearly improve insulin resistance and control blood glucose for a long term (Mao et al., 2002). Because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries, so plants are used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease (Djomeni et al., 2006). In alternative medication, mahkota dewa (Phaleria macrocarpa [Scheff.] Boerl) fruits has been reported to be useful as effective remedies against diabetes, cancer, gout, high cholesterol, and kidney disorder (Harmanto, 2005). The hypoglycemic activity of isolate from ethyl acetate fraction of mahkota dewa fruits has been reported (Sari, 2004).

In the present study, the activity of isolate from ethyl acetate fraction of mahkota dewa fruits on insulin sensitivity in hyperglycemic mouse was evaluated. The result could be the basic for the development of mahkota dewa fruits as antihyperglycemic.

Materials and Methods

Chemicals

Aquadest, ethanol 70%, ethyl acetate, n-Hexane, silica gel for column 1.07734.1000 (70-230 mesh), sulphate acid, glucose, exogenous insulin (Humulin®, PT Tempo Scan Pacific Tbk.), metformin® (PT Dexta Medica), Pulvis Gummi Arabicum (PGA).

Animals

Male white mice (Mus musculus), 3-4 weeks old, 20-30 gram weight, provided by Animal Breeding Laboratory, Education University of Indonesia.

Plant material and preparation of extract and fraction and isolation

1. Extraction

Mahkota dewa (Phaleria macrocarpa [Scheff.] Boerl.) fruits were macerated with ethanol 70% for 3x24 hours. Then the macerate was concentrated by rotary evaporator until it’s thick (PM).
2. Fractionation

The thick extract (PM) was fractionated by liquid-liquid extraction in funnel based on the gradient polarity. The solutions that were used are aquaest, n-Hexane, and ethyl acetate.

**Results and Discussion**

### Results of extraction, fractionation, and isolation

Results of extraction, fractionation, and isolation are shown in Table 1. Isolate PM-IIAy1 has the characteristic of purple colour in visible and white-purple fluorescence in UV (λ=366 nm) after sprayed with H$_2$SO$_4$, and value of Rf was 0,5 on Thin Layer Chromatography (TLC) with eluents were n-Hexane:ethyl acetate (7:3).

### Evaluation on insulin sensitivity of isolate from ethyl acetate fraction of mahkota dewa fruits

Insulin resistance is hard to be measured. The standard way is with insulin clamp technique to the animals by measuring the rate of glucose that’s injected intravenously to endure euglycemic condition while insulin was infused. Decreasing of insulin sensitivity is shown if high insulin necessity in control the normoglycemic condition (Merentek, 2006).

The graph of the rate of glucose level of mouse on insulin sensitivity evaluation of isolate PM-IIAy1 is shown in Figure 1. Those rates of glucose level was analyzed statistically with student’s t test to shows the differences of insulin sensitivity based on the insulin capability in decreasing blood glucose level that compared on negative control. The results of analysis are shown in Table 2.

Statistical evaluation (α=0,05) results that there’s no differences significantly of fasting blood glucose level in negative control, positive control, and study’s group. High blood glucose levels at 0’s minute in these groups means the decreasing of insulin sensitivity that’s shown by inability of endogenous insulin in controlling to normoglycemic condition, thus the fasting blood glucose level are high or hyperglycemic. To evaluate the insulin sensitivity after the treatment in seven days, all mouse were injected of glucose and exogenous insulin in the vein, to know the ability of insulin to decreasing blood glucose level after glucose injection.

At the 3rd minute after glucose and insulin injection, there’s the increasing of blood glucose level in all groups. The results of student’s t test showed that there were no differences significantly between study’s group and negative control although the blood glucose level of study’s group are lower than negative control group. But they also showed that there were no differences significantly between study’s group and positive control group. Those mean that insulin couldn’t control the increasing of blood glucose level.

At the 6th minute, the differences of decreasing blood glucose level in all groups had been shown by the work of insulin. The study’s group showed a significant difference with negative control group. It means isolate PM-IIAy1 had an activity in increasing insulin sensitivity that’s shown by the ability of insulin decreasing blood glucose level lower than negative control. It caused by insulin can facilitated
the uptake of glucose by cell. But, the activity of isolate PM-IIAy1 had not been as well as metformin yet, showed in the differences significantly at this minute.

At the 9th minute, the study’s group showed the difference significantly with negative control, and had no difference with positive control. It means isolate PM-IIAy1 had improved the insulin sensitivity rather than the minutes before because had shown a similar activity with metformin. It showed continuously till the 15th minute. But at the 18th minute, the study’s group had shown no difference significantly with negative control. It means isolate PM-IIAy1 showed no activity anymore.

These results of the evaluation of isolate PM-IIAy1 on insulin sensitivity showed that isolate PM-IIAy1 has an activity in increasing insulin sensitivity in hyperglycemic mouse.

The percentages of the raising of insulin sensitivity are calculated that compared to negative control. The raising of insulin sensitivity of isolate PM-IIAy1 at 3rd, 6th, 9th, 12th, 15th, and 18th minutes are 13.04 %, 13.29 %, 18.3 %, 15.03 %, 15.94 % and 12.60 %, while the percentages of metformin are 23.84 %, 20.10 %, 23.84 %, 16.56 %, 19.81 % and 17.32 %. The bar chart of the percentages of insulin sensitivity increment of isolate PM-IIAy1 and metformin toward to negative control group is shown in Figure 2.

Table 1 Rendemen of the extraction, fractionation, and isolation

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Crude plant</th>
<th>PM</th>
<th>PM-IIA</th>
<th>PM-IIAy</th>
<th>PM-IIAy1</th>
</tr>
</thead>
<tbody>
<tr>
<td>691.24</td>
<td>136.31</td>
<td>30</td>
<td>1.12</td>
<td>0.1115</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>19.72</td>
<td>4.34</td>
<td>0.16</td>
<td>0.016</td>
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</tbody>
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Table 2 Rate of mice blood glucose level (mg/dL) on insulin sensitivity evaluation of isolate PM-IIAy1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rate of blood glucose level (mg/dL)</th>
<th>t (min) 0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
<th>15</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td></td>
<td>127.00</td>
<td>237.67</td>
<td>195.67</td>
<td>180.00</td>
<td>153.00</td>
<td>138.00</td>
<td>127.00</td>
</tr>
<tr>
<td>Normal control</td>
<td></td>
<td>72.00*</td>
<td>124.33*</td>
<td>104.67*</td>
<td>89.33*</td>
<td>76.33*</td>
<td>68.00*</td>
<td>65.33*</td>
</tr>
<tr>
<td>Positive control</td>
<td></td>
<td>111.00</td>
<td>181.00*</td>
<td>156.33*</td>
<td>140.33*</td>
<td>127.67*</td>
<td>110.67*</td>
<td>105.00*</td>
</tr>
<tr>
<td>Test</td>
<td></td>
<td>115.67</td>
<td>206.67</td>
<td>169.67*</td>
<td>147.00*</td>
<td>130.00*</td>
<td>116.00*</td>
<td>108.67</td>
</tr>
</tbody>
</table>

* P<0.05 vs negative control
** P<0.05 vs negative control and positive control

Information :
Normal control : PGA 2%
Negative control : PGA 2% + glucose 5.35 g/kg BW
Positive control : PGA 2% + metformin 143 mg/kg BW + glucose 5.35 g/kg BW
Test group : PGA 2% + isolate PM-IIAy1 143 mg/kg BW + glucose 5.35 g/kg BW

Figure 1 Rate of blood glucose level (mg/dL) of mice in evaluation on insulin sensitivity of isolate PM-IIAy1
Figure 2  Percentages of insulin sensitivity increment in evaluation on insulin sensitivity of isolate PM-IIAy1 and metformin toward to negative control

Conclusions

The research and analysis indicated that isolate from ethyl acetate fraction of mahkota dewa fruits (PM-IIAy1) could improve insulin sensitivity in hyperglycemic mice toward to negative control. The highest activity of insulin sensitivity increment was 18.3 % on the 9th minute. Activity of isolate PM-IIAy1 wasn’t different significantly with metformin.

References


