INSULIN LEAVES (SMALLANTHUS SONCHIFOLIUS) DRY EXTRACT IMPROVES BLOOD GLUCOSE AND LIPID PROFILE IN ALOXAN-INDUCED RAT

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Abstract - Insulin leaves (Smallanthus sonchifolius) have been traditionally used in South America as an anti-hyperglycemic agent, however, the dose, the usage and the duration of these leaves as an anti-hyperglycemic agent is unclear. This work intends to elucidate the impact of insulin leaves dry extract on blood glucose and lipid profile in aloxan-induced rat. Insulin leaves were extracted as dry extract. Aloxan-induced Sprague-Dawley rat were given 300 mg/kg BW insulin leaves dry extract once daily for 14 days. Blood glucose level and body weight were evaluated weekly. Cholesterol level and tryglyceride level were analyzed at the end of the study. Aloxan injection induced hyperglycemic condition in the rat, mimicking diabetic condition. Daily decoction of insulin leaves dry extract for 14 days significantly reduced blood glucose level in aloxan-induced rats. Additionally, cholesterol and tryglyceride level were increased in aloxan-induced rats and daily decoction of insulin leaves dry extract for 14 days significantly reduced these levels. Fourteen days daily decoction of 300 mg/kg BW insulin leaves dry extract improved blood glucose and lipid profile in diabetic rat, suggesting the further potential of insulin leaves as diabetic drug.

INTRODUCTION

Type 1 diabetes mellitus (DM) is associated with the inhibition of insulin secretion from pancreatic β-cells, thus the tissues do not adequately metabolize the glucose, resulting in chronic and progressive hyperglycemia (World Health Organization, 2009). Several studies have shown that hyperglycemia further lead to renal (Garud and Kulkarni, 2014; Elseweidy, 2014; Kitada, et al., 2014), cardiac (Cai, et al., 2002; Lei, et al., 2013) and ocular complications (Kim, et al., 2014) by activating many cellular pathways, suggesting glycemic control as the most important strategy in DM. Anti-diabetic agents of natural products are recommended by WHO to control glycemic condition and prevent diabetic complications (World Health Organization, 2009).

Smallanthus sonchifolius, popularly known as yacon, is a native plant to South America and has been traditionally used as anti-diabetic agents (Lachman, et al., 2003; Valentova, et al., 2006). Recently, yacon has gained increasing popularity in Indonesia for its low glycemic value, mimicking insulin agent, so that they called yacon as daun insulin (insulin leaves). Several studies have shown that parts of yacon, including roots (Oliveira, 2013) and leaves (Honore, et al., 2012; Genta, et al., 2010), give benefit in DM by reducing hyperglycemia and prevent from further complications. Moreover, Habib et al., (2011) have reported that yacon root flour, a natural product rich in fructooligosaccharides (FOS), gave beneficial effect on diabetes-associated hyperlipidemia by reducing fasting plasma triacylglycerol and low-density lipoprotein (LDL). Various preparations including roots aqueous extract (Oliveira, et al., 2013), methanol extract (Genta, et al., 2010), buthanol extract (Genta, et al., 2010), chloroform extract (Genta, et al., 2010), yacon flour (Habib, et al., 2011), 10% yacon solution (Honore, et al., 2012) and yacon...
diet (Satoh et al., 2013) have been published to give beneficial effect on diabetic with various doses. Accordingly, in the present study, we took advantage to assess the effect of 14 days daily decoction of *Smallanthus sonchifolius* leaves dry extract on ameliorating hyperglycemia and diabetes-associated hyperlipidemia.

**MATERIALS AND METHODS**

**Animals and induction of diabetes**

Male Sprague-Dawley rats weighing between 200 and 240 g were obtained from iRatco Inc, Bogor, Indonesia. The rat were allowed to acclimatize to the local vivarium and allowed free access to standard laboratory diet and drinking water. Diabetes was induced by a single intraperitoneal injection (125 mg/kg body weight) alloxan monohydrate (Sigma-Aldrich, Inc., St. Louis, MO, USA) dissolved in distilled water. The rats were considered diabetic and used for the study only if they had hyperglycemia (=300 mg/dL) at 5 days after alloxan injection. Animal protocols were carried out with approval from the Review Board for animal studies at our institutes. All procedures were performed under minimal suffering.

*Smallanthus sonchifolius* (daun insulin) extraction

*Smallanthus sonchifolius* (daun insulin) leaves were determined by Centre for Plant Conservation, Bogor Botanical Gardens, Indonesian Institute of Sciences. Further, the leaves were extracted as dry extract in Bogor Agricultural University.

**Experimental protocol**

Five days after alloxan injection, diabetic rats were randomly selected and divided into two groups, namely, diabetic (D, n = 4) and diabetic treated with *Smallanthus sonchifolius* (D + SS, n = 4). Normal rats were randomy selected and divided into two groups, as normal control (N, n = 5) and as normal vehicle control (treated with *Smallanthus sonchifolius*, (N + SS), n= 5). For 14 days, the N and D group received distilled water, the vehicle of *Smallanthus sonchifolius*, whereas the N + SS and D + SS groups received a suspension of *Smallanthus sonchifolius* orally with the dose 300 mg/kg body weight. Body weight and blood glucose level were measured on the time before receiving treatment (day 1), day 7 and on the sacrifice day (day 14). Blood samples were drawn from vena cava inferior in each rat at the time of sacrifice into EDTA vacutainer tubes. EDTA-blood was centrifuged at 3000g, 4°C, for 15 min for separation of plasma.

**Lipid profile analysis**

Plasma cholesterol was measured by the cholesterolesterase/cholesterol oxidase technique kit analysis (Sclavo Diagnostics, Siena, Italy). Likewise, plasma tryglyceride was measured enzymatically by kit (Sclavo Diagnostics, Siena, Italy).

**Statistical analysis**

Data are presented as mean and standard error of mean (S.E.M). Comparisons among groups were performed using the one-way analysis of variance (ANOVA) followed by Tukey’s method for post hoc analysis and t-test, wherever applicable. Differences were considered statistically significant at probability values < 0.05.

**RESULTS**

**Blood glucose level and body weight changes**

Five days after alloxan monohydrate injection, blood glucose level were significantly increased in rats. These rats were randomly allocated in the D group (n = 5) as positive control and in the D + SS group (n = 5) as treatment group. The average level was more than 300 mg/dL (Table 1). Additionally, the body weight was decreased in all groups on D7, however, permanent decrease was observed only in the D group (Table 1).

**Effect of Smallanthus sonchifolius on blood glucose level, body weight and lipid profile**

As shown in Table 2, *Smallanthus sonchifolius* treatment did not affect body weight of normal rats in the N + SS group, however, it suppressed a reduction in the body weight within the D + SS group, although this effect was not significant compared with that of the D group. The average blood glucose level was more than 300 mg/dL in the D group and remained at this level during the experimental period of 14 days. *Smallanthus sonchifolius* treatment significantly decreased blood glucose level in the D + SS group compared with that of the D group, although the blood glucose level did not reach normal level compared with the N group (Table 2). Furthermore, significant increases of plasma cholesterol and tryglycerides concentration were observed in the D group fourteen days after alloxan monohydrate injection.
Smallanthus sonchifolius treatment significantly decreased plasma cholesterol and tryglycerides concentration in the D + SS group compared with that of the D group.

**DISCUSSION**

Diabetes is a condition characterized by chronic and progressive hyperglycemia which causes metabolic abnormalities and may lead to further complications. Ameliorating hyperglycemia has become the main strategy in the diabetes treatment. In this present study, Smallanthus sonchifolius was investigated for its ability to ameliorate hyperglycemia and further prevent diabetes-associated hyperlipidemia. Several studies have shown that daily decoction of Smallanthus sonchifolius leaves dry extract (with the dose of 70 mg/kg BW and 400 mg/kg BW) gave beneficial role in the diabetic animal model by reducing blood glucose level (Honore, 2012; Baroni, 2008), however, prolonged consumption of Smallanthus sonchifolius may also lead to renal toxicities (de Oliveira, 2011), thus several studies are needed in order to find the optimum dose, time and preparation for diabetes treatment. Our results have shown that 14 days daily decoction of 300 mg/kg BW Smallanthus sonchifolius leaves dry extract significantly reduced blood glucose level compared to those in the untreated rats. Possible explanation for hypoglycemic effect of Smallanthus sonchifolius leaves is that active compound of Smallanthus sonchifolius leaves, the smallanthaditerpenic acids A, B, C and D exert anti-diabetic properties through HPLC analysis (Xiang, 2010). Hypoglycemic effect of Smallanthus sonchifolius is observed not only in the leaf but also in the root. Satoh (2013) have reported that Smallanthus sonchifolius diet on Zucker fa/ta rats improves hepatic insulin resistance by reducing treble 3 (TRB3) expression, a pseudokinase induced during diabetic condition. Moreover, 90

### Table 1. Changes in blood glucose level and body weight of rats induced by alloxan monohydrate.

<table>
<thead>
<tr>
<th></th>
<th>D1</th>
<th>D7</th>
<th>D14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose level (mg/dL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>133.4 ± 7.01</td>
<td>118 ± 11.75</td>
<td>130.6 ± 8.11</td>
</tr>
<tr>
<td>N + SS</td>
<td>131.8 ± 7.04</td>
<td>117.8 ± 11.8</td>
<td>137 ± 5.3</td>
</tr>
<tr>
<td>D</td>
<td>540.25 ± 27.31**</td>
<td>430 ± 110.38*</td>
<td>536.25 ± 42.46**</td>
</tr>
<tr>
<td>DD + SS</td>
<td>487.25 ± 68.79**</td>
<td>407.25 ± 106.96*</td>
<td>345.75 ± 59.61**#</td>
</tr>
<tr>
<td>Body weight (percentage)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>100</td>
<td>91.79 ± 2.15</td>
<td>103.67 ± 8.36</td>
</tr>
<tr>
<td>N + SS</td>
<td>100</td>
<td>89.01 ± 4.04</td>
<td>103 ± 8.78</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>96.75 ± 11.03</td>
<td>98.32 ± 6.78</td>
</tr>
<tr>
<td>DD + SS</td>
<td>100</td>
<td>96.42 ± 7.68</td>
<td>107.73 ± 4.49</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M.
N, normal rats; N + SS, normal rats administered with Smallanthus sonchifolius; D, diabetic rats administered with vehicle; D + SS, diabetic rats administered with Smallanthus sonchifolius.
D1, day 1; D7, day 7; D14 day 14 from administration of Smallanthus sonchifolius and vehicle.
* p < 0.05 and ** p < 0.01 vs. N ; # p < 0.05 and ## p < 0.01 vs. D

### Table 2. Changes in biochemical parameters after 14 days of treatment with Smallanthus sonchifolius in diabetic rats induced by alloxan monohydrate

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>N + SS</th>
<th>D</th>
<th>D + SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight changes (%)</td>
<td>103.67 ± 8.36</td>
<td>103 ± 8.78</td>
<td>98.32 ± 6.78</td>
<td>107.73 ± 4.49</td>
</tr>
<tr>
<td>Blood glucose level (mg/dL)</td>
<td>130.6 ± 8.11</td>
<td>137 ± 5.3</td>
<td>536.25 ± 42.46**</td>
<td>345.75 ± 59.61##</td>
</tr>
<tr>
<td>Cholesterol level (mg/dL)</td>
<td>98.8 ± 22.72</td>
<td>135.91 ± 10.78</td>
<td>264.28 ± 45.77*</td>
<td>115.33 ± 22.89#</td>
</tr>
<tr>
<td>Tryglycerides level (mg/dL)</td>
<td>115.33 ± 17.03</td>
<td>85 ± 6.88</td>
<td>137.72 ± 13.31**</td>
<td>48.16 ± 2.26##</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M.
N, normal rats; N + SS, normal rats administered with Smallanthus sonchifolius; D, diabetic rats administered with vehicle; D + SS, diabetic rats administered with Smallanthus sonchifolius.
* p < 0.05 and ** p < 0.01 vs. N ; # p < 0.05 and ## p < 0.01 vs. D
days consumption of *Smallanthus sonchifolius* roots flour that rich of fructooligosaccharides increased the insulin-positive pancreatic cell mass & reduced fasting plasma triacylglycerol and LDL via the regulation of glucagon like peptide (GLP)-1 (Habib, 2011). We further found that *Smallanthus sonchifolius* leaves dry extract significantly reduced plasma cholesterol and tryglycerides level compared to those in the untreated rats, however, the exact mechanisms should be elucidated since fructooligosaccharides are present only in the root but not the leaf.

In this study, we conclude that 14 days daily decoction of 300 mg/kg BW *Smallanthus sonchifolius* leaves dry extract attenuated high blood glucose level and improved diabetes-associated hyperlipidemia in diabetic condition, suggesting further potential of *Smallanthus sonchifolius* leaves as anti-diabetic drug.

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REFERENCES


